Epidemiology of lumpy jaw in captive macropods across Australia and Europe: An investigation of disease risk and treatment approaches

Jessica Amy Jane Rendle

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MSc Animal Welfare

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“It always seems impossible until it’s done.”

Nelson Mandela

AUTHOR'S DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Jessica Rendle

PhD Candidate

March 2019

ABSTRACT

Lumpy jaw is a well-recognised cause of morbidity and mortality in captive macropods (Macropodidae) worldwide. The extent and causes of the disease are largely unknown, although multiple risk factors associated with a captive environment are thought to contribute to the development of clinical disease. Identification of risk factors associated with lumpy jaw would assist with the development of preventive management strategies, potentially reducing mortalities.

A cross-sectional study was undertaken from 2011 to 2015, to determine prevalence and risk factors for this disease through the distribution of a survey to 527 institutions across Australia and Europe; two regions where macropods are popular exhibits. Veterinary and husbandry records from the period 1st January 1995 up to and including 28th November 2016 (the last date when data were extracted from zoo records) were analysed in a retrospective cohort study, examining risk factors for developing disease and treatments used, over time. Computed tomography was used to examine disease occurrence in wild macropods using skulls from population management culls.

The prevalence of lumpy jaw was found to differ between the two regions ($p <$ 0.0002). A review of 6178 records for 2759 macropods housed within eight zoos across the Australian and European regions, found incidence rates and risk of infection differed between geographic regions and individual institutions. Risk of developing lumpy jaw increased with age, particularly for macropods >10 years (Australia IRR 7.63, $p < 0.001$; Europe IRR 7.38, $p < 0.001$). Treatment approach varied and prognosis was typically poor with 62.5% mortality for Australian and European regions combined. Lumpy jaw was detected in all captive genera examined, but was absent from the wild populations studied.

Geographic region influenced the incidence of lumpy jaw, the risks associated with developing clinical disease, and preferred treatment approach. Despite advances in antibiotic therapy and surgical techniques, treatment of lumpy jaw is largely

unrewarding for the individual and should be approached on an individual basis. This research provides new information about this refractory disease and makes practical recommendations to reduce disease risk. This information may assist institutions in providing optimal long-term health management for captive macropods; such efforts having a positive impact on both welfare and conservation, including but not limited to captive breeding and translocation programs.

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GLOSSARY OF ABBREVIATIONS AND TERMS

AIPMMA beads	Antibiotic impregnated polymethylmethacrylate beads
AST	Aspartate aminotransferase
AZA	American Zoo Association
BIAZA	British and Irish Association of Zoos and Aquariums
bid	Medication twice a day
blepharospasm	Neurological closing of the eyelids
BSE	Bovine Spongiform Encephalopathy
c	Captive
C	Canine
CEO	Chief Executive Officer
CI	Confidence interval
CK	Creatine kinase
CR	Critically endangered
CT	Computed Tomography
DEC	Department of Environment and Conservation
Dendrolagus bennettianus	Bennett's tree kangaroo
Dendrolagus dorianus	Doria's tree kangaroo
Dendrolagus goodfellowi	Goodfellow's tree kangaroo
Dendrolagus inustus	Grizzled tree kangaroo
Dendrolagus lumholtzi	Lumholtz's tree kangaroo
Dendrolagus matschiei	Matschie's tree kangaroo
Dendrolagus spadix	Lowland tree kangaroo
Dendrolagus stellarum	Seri's tree kangaroo
Dendrolagus stottae	Tenkile tree kangaroo
Dendrolagus ursinus	Vogelkop tree kangaroo
Dorcopsis atrata	Black dorcopsis
Dorcopsis hageni	White-striped dorcopsis
Dorcopsis luctuosa	Grey dorcopsis
Dorcopsis muelleri	Brown dorcopsis

Dorcopsulus macleayi	Macleay's dorcopsis
Dorcopsulus vanheurni	Small dorcopsis
dysphagia	Difficulty swallowing
EAZA	European Association of Zoos and Aquaria
EN	Endangered
f	Female
GAN	Global accession number
halitosis	Bad breath
HPA	hypothalamic pituitary adrenal axis
hr	Per hour
hyperptyalism	Increased secretion of saliva/drooling
I	Incisor
IM	Intramuscular
Incidence	The number of new cases that occur in a population (at risk) over a specified time period.
Incidence rate (IR)	The number of new cases of LJ in a specified time period, divided by the animal-time at risk.
Incidence rate ratio (IRR)	Incidence rate ratio is the ratio of two incidence rates.
IR	Incidence rate
IRR	Incidence rate ratio
ISIS	International Species Information System (now Species360)
iu	International units
IUCN	International Union for Conservation of Nature
IV	Intravenous
kg	Kilogram
LA	Long acting (antibiotic)
Lagorchestes conspicillatus	Spectacled hare wallaby
Lagorchestes hirsustus	Rufous hare wallaby
LC	Least concern
LJ	Lumpy jaw

m	Male
M	Molar
M	Mean
Macropus agilis	Agile wallaby
Macropus antilopinus	Antilopine wallaroo/kangaroo/wallaby
Macropus bernardus	Black wallaroo
Macropus dorsalis	Black-striped wallaby
Macropus eugenii	Tammar wallaby
Macropus fuliginosus	Western grey kangaroo
Macropus giganteus	Eastern grey kangaroo
Macropus irma	Western brush wallaby
Macropus parma	Parma wallaby
Macropus parryi	Whiptail wallaby
Macropus robustus	Common wallaroo
Macropus rufogriseus	Red-necked (Bennett's) wallaby
Macropus rufus	Red kangaroo
Merycism	Regurgitation activity undertaken by some macropods
mg	Milligram
MIA	Maximum Avoidance Inbreeding schemes
Mulch	Shredded plant material
n	Denominator
NT	Near threatened
Onychogalea fraenata	Bridled nail-tail wallaby
Onychogalea unguifera	Northern nail-tail wallaby
OR	Odds ratio
Osteolysis	Pathological destruction or disappearance of bone tissue
Osteomyelitis	Infection of the bone
P	Prevalence
p	*p*-value, level of significance

Period prevalence	The proportion of the population positive for disease in the given time period. The sum of the point prevalence at the beginning of the period, and the number of new cases that occur during that period.
Petrogale assimilis	Allied rock wallaby
Petrogale brachyotis	Short-eared rock wallaby
Petrogale burbidgei	Monjon
Petrogale coenensis	Cape York rock wallaby
Petrogale concinna	Narbelek
Petrogale godmani	Godman’s rock wallaby
Petrogale herberti	Herbert’s rock wallaby
Petrogale inornata	Unadorned rock wallaby
Petrogale lateralis	Black-footed rock wallaby
Petrogale mareeba	Mareeba rock wallaby
Petrogale penicillata	Brush-tailed rock wallaby
Petrogale persephone	Proserpine rock wallaby
Petrogale purpureicollis	Purple-necked rock wallaby
Petrogale rothschildi	Rothschild’s rock wallaby
Petrogale sharmani	Sharman’s rock wallaby
Petrogale xanthopus	Yellow-footed rock wallaby
Pleistocene	Period of time from 2.6 million years ago until about 11,700 years ago.
PM	Pre-molar
PMMA	Polymethylmethacrylate beads
PO	Taken orally
Point prevalence	The proportion of disease in a population at a particular point in time.
Prevalence	The proportion of animals affected with disease in a known population at a designated time. There is no distinction between old and new cases.
PY	Pouch young

q	Every,each
SC	Subcutaneous
SD	Standard deviation
Se	Sensitivity
Setonix brachyurus	Quokka
SHR	Species holding report
Sp	Specificity
sp.	Species – singular/plural
spp.	Species - plural
Species360	Global zoological membership-based organisation (formerly International Species Information System [ISIS])
TAG	Taxon Advisory Group
Thylogale billardierii	Tasmanian pademelon
Thylogale browni	New Guinea pademelon
Thylogale brunii	Dusky pademelon
Thylogale calabyi	Calaby's pademelon
Thylogale lanatus	Mountain pademelon
Thylogale stigmatica	Red-legged pademelon
Thylogale thetis	Red-necked pademelon
TMJ	Temporomandibular joint
ud	Undetermined
UK	United Kingdom
USA	United States of America
VAG	Veterinary Advisory Group
VetSAG	Veterinary Specialist Advisory Group
VU	Vulnerable
w	Wild
WA	Western Australia
Wallabia bicolor	Swamp wallaby
WAZA	World Association for Zoos and Aquariums
x	Numerator

ZAA	Zoo and Aquarium Association (Australia)
ZIMS	Zoological Information Management System

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CHAPTER 1

General Introduction

1.1 General overview

Lumpy jaw is a well-recognised cause of morbidity and mortality in captive macropods worldwide (Wallach, 1971; Butler & Burton, 1980; Samuel, 1983; Vogelnest & Portas, 2008; Borland et al., 2012). It is a disease of multifactorial aetiology (Smith et al., 1986; Vogelnest & Portas, 2008), and multiple risk factors associated with captive management are hypothesised to contribute to the development of clinical disease (Vogelnest & Portas, 2008; Kido et al., 2013). Yet despite suggestion of its common occurrence, there is limited, and often dated, scientific evidence of the risks associated with the development of lumpy jaw or the geographical distribution of the disease across zoological institutions.

Lumpy jaw is a major cause of concern for zoo veterinarians and macropod keepers, not only due to welfare issues for the individual animals affected, but also due to the complexity of disease treatment, frequent recurrence, and anticipated low survival (Lewis et al., 1989; Vogelnest & Portas, 2008). The provision of treatment is therefore considered likely to be unrewarding for the animal and subsequently costly to the zoo. Given this, there would be benefits to identifying specific factors associated with incidence of lumpy jaw in captivity, which could assist with the development of preventive management strategies. The continued recurrence of lumpy jaw in captive macropod populations enables epidemiological investigations of host and environmental risk factors for the disease to be undertaken, and these are fundamental to the development of recommendations for disease management and improvements in captive macropod welfare.

Epidemiological studies are useful for observing trends in the occurrence of disease and to validate risk factors associated with the development of disease (Thrusfield & Christley, 2018). To systematically examine disease occurrence, duration, climatic trends in incidence, treatments, recurrence and survival, a cross-sectional survey and a retrospective cohort study were designed, to investigate epidemiology of lumpy jaw across two regions where macropods are popular exhibits: Australia and Europe. Here we present the findings from these epidemiological investigations of lumpy jaw in captive macropods.

1.2 The macropod

1.2.1 Taxonomic classification of macropods

The term 'macropod' is used to collectively describe kangaroos, wallabies and their relatives that are part of the Superfamily Macropodoidea (Gray, 1821). Collectively, Macropodoidea contains three Families, including Macropodidae; for which the taxonomy is further broken down to Subfamily level, which includes the Macropodinae (Gray, 1821). This Subfamily comprises of subjects of this present study, and encompasses 10 genera and at least 62 extant species endemic to Australia, New Guinea and surrounding islands (Strahan, 1995; Woinarski et al., 2014). In this study, we use the term 'macropod' to describe all genera of kangaroo, wallaby and quokka in the Subfamily Macropodinae (Thomas, 1888), including *Dendrolagus*, *Dorcopsis*, *Lagorchestes*, *Macropus*, *Onychogalea*, *Petrogale*, *Thylogale*, *Setonix* and *Wallabia*. Changes to taxonomic classification may result in reclassification of some species and genera; therefore, the classification selected for use in this research was that which was in use at the commencement of this research. Although several subspecies have been described in the literature, recent morphological and genetic studies have raised questions about the validity of some subspecies differences (Neaves et al., 2012; Woinarski et al., 2014); therefore, all taxonomic classifications in this study will remain at or above the species level.

1.2.2 Natural history

Macropods are distinguished by size, with the kangaroos being the largest of the macropods, the wallabies being smaller, and the wallaroos spanning somewhere in between (Dawson, 1995; Vogelnest & Portas, 2008). There are also behavioural differences between macropods, with the larger kangaroo species being more gregarious, such as the red kangaroo (*Macropus rufus*), whilst many wallabies are non-gregarious, leading a more solitary lifestyle, such as the parma wallaby (*M. parma*) (Kaufmann, 1974; Dawson, 1995; Coulson, 1997; Ord et al., 1999). Anatomical and behavioural differences between macropods are often related to differences in their habitat and herbivorous diets (Lee & Ward, 1989; Clancy & Croft, 1991; Dawson, 1995; Warburton, 2009; Arman & Prideaux, 2015).

1.2.3 Geographical distribution and habitat

Wild macropods occur throughout Australia and Papua New Guinea (Flannery, 1995; Strahan, 1995; Flannery et al., 1996). Habitats in which they are found vary considerably; from arid desert, to coast, to sclerophyll forests, and from rainforest in the north, to alpine forests in Tasmania (Jarman, 1984; Clancy & Croft, 1989; Flannery, 1995; Strahan, 1995; Flannery et al., 1996; Van Dyck & Strahan, 2008).

1.2.4 Conservation status

Australia's endemic animals, including macropods, have been in decline since European settlement (Woinarski et al., 2015). Within Macropodidae, at least four macropod species have become extinct since 1788 (Woinarski et al., 2015), and a further 12 are classified by the International Union for Conservation of Nature (IUCN) as endangered, four of which are critically endangered (International Union for Conservation of Nature, 2016) (Table 1.1).

1.2.5 Macropod biology

Variation in the morphometrics of macropods can be observed across the Macropodidae family, with some wallabies weighing <1kg, for instance, the rufous hare wallaby (*Lagorchestes hirsutus*), whilst species of kangaroo, such as the red kangaroo, can reach 90 kg (Table1.1). The distinct morphometrics of macropods are discussed in Strahan (1995), Dawson (1995) and Flannery et al. (1996). There are also biological distinctions within some macropod species, with some species expressing sexual dimorphism (Newsome et al., 1977; Dawson, 1995). There are distinct dimorphic differences in size and colour in the red kangaroo (Dawson, 1995). Sexual dimorphism may also be observed in the dental development of some macropod species, for example, the agile wallaby (*Macropus agilis*) (Newsome et al., 1977).

Table 1.1: Conservation status, longevity in captivity (c) and the wild (w), and weight range for extant macropod species (Macropodinae).

Genus	**Species common name**	**IUCN status**	**Longevity (c) (w)**	**Weight (kg)**
Dendrolagus	Bennett's tree kangaroo	NT	6 - 20 (c)	13
(Tree kangaroo)	Dingiso	EN	-	-
	Doria's tree kangaroo	VU	19.0 (c)	-
	Golden-mantled tree kangaroo	CR	-	-
	Goodfellow's tree kangaroo	EN	23.6 (c)	-
	Grizzled tree kangaroo	VU	23.8 (c)	-
	Ifola tree kangaroo	EN	-	-
	Lowland tree kangaroo	VU	-	-
	Lumholtz's tree kangaroo	NT	-	3.7 - 10.0
	Matschie's tree kangaroo	EN	26.9 (c)	-
	Seri's tree kangaroo	VU	-	-
	Tenkile tree kangaroo	CR	-	-
	Vogelkop tree kangaroo	VU	20.0 (c)	-
	Wondiwoi tree kangaroo	CR	-	-
Dorcopsis	Black dorcopsis	CR	-	-
(Forest wallaby, dorcopsis)	Brown dorcopsis	LC	12.4 (c)	-
	Grey dorcopsis	VU	13.9 (c)	-
	White-striped dorcopsis	LC	-	-
Dorcopsulus	Macleay's dorcopsis	LC	7.9 (c)	-
(Dorcopsis)	Small dorcopsis	NT	-	-
Lagorchestes	Rufous hare wallaby	VU	13.2 (c)	0.8 - 2.0
(Hare wallaby)	Spectacled hare wallaby	LC	7.0 - 13.0 (c)	1.6 - 4.6
Macropus	Agile wallaby	LC	16.9 (c) 14 (w)	9.0 - 27.0
(Wallaby, kangaroo, wallaroo)	Antilopine wallaroo	LC	19.8 (c)	16.0 - 49.0
	Black wallaroo	NT	11.8 (c)	13.0 - 22.0
	Black-striped wallaby	LC	12.4 (c) >15.0 (w)	6.0 - 20.0
	Common wallaroo	LC	22.0 (c)	6.3 - 46.5
	Eastern grey kangaroo	LC	25.0 (c) 20.0 (w)	3.5 - 66.0
	Parma wallaby	NT	15.9 (c) 8.0 (w)	3.2 - 5.9
	Red kangaroo	LC	25.0 (c)	17.0 - 90.0
	Red-necked (Bennett's) wallaby	LC	19 (c) 18.6 (w)	11.0 - 27.0
	Tammar wallaby	LC	15.1 (c) 14.0 (w)	4.0 - 10.0

Genus	Species common name	IUCN status	Longevity (c) (w)	Weight (kg)
	Western brush wallaby	LC	-	7.0 - 9.0
	Western grey kangaroo	LC	23.2 (c) >20.0 (w)	3.0 - 54.0
	Whiptail wallaby	LC	10.0 - 14.0 (w)	7.0 - 26.0
Onychogalea	Bridled nail-tail wallaby	VU	6.0 - 10.0 (c)	4.0 - 6.0
(Nail-tail wallaby)	Northern nail-tail wallaby	LC	5.5 - 10.0 (c)	4.5 - 9.0
Petrogale (Rock wallaby)	Allied rock wallaby	LC	6.0 - 15.0 (c) 8.0 - 15.0 (w)	4.4
	Black-footed rock wallaby	VU	9.3 - 12.0 (c)	4.6
	Brush-tailed rock wallaby	VU	14.3 (c)	5.8 - 7.5
	Cape York rock wallaby	NT	-	4.0 - 5.0
	Godman's rock wallaby	NT	-	5
	Herbert's rock wallaby	LC	-	-
	Mareeba rock wallaby	NT	-	3.8 - 4.5
	Monjon	NT	-	1.0 - 1.4
	Mount Claro rock wallaby	VU	-	3.7
	Narbelek	EN	11.7 (c)	1.2
	Proserpine rock wallaby	EN	-	5.0 - 8.0
	Purple-necked rock wallaby	NT	-	5.7
	Rothschild's rock wallaby	LC	-	5.3
	Short-eared rock wallaby	LC	10.1 (c)	3.7 - 4.5
	Unadorned rock wallaby	LC	-	4.7
	Yellow-footed rock wallaby	NT	14.4 (c)	6.0 - 7.0
Setonix (Quokka)	Quokka	VU	6.0 - 10.0 (c)	2.7 - 4.2
Thylogale	Calaby's pademelon	EN	-	-
(Pademelon)	Dusky pademelon	VU	9.4 (c)	-
	Mountain pademelon	EN	-	-
	New Guinea pademelon	VU	-	-
	Red-legged pademelon	LC	9.7 (c)	3.7 - 6.8
	Red-necked pademelon	LC	9.0 (c)	1.8 - 9.1
	Tasmanian pademelon	LC	4 - 8 (c)	2.4 - 12.0
Wallabia bicolor (Swamp wallaby)	Swamp wallaby	LC	5.0 - 9.0 (c)	10.3 - 20.6

CR – Critically endangered; EN – Endangered; LC – Least concern; NT – Near threatened; VU – Vulnerable (Flannery, 1995; Flannery et al., 1996; Nowak & Walker, 1999; Fisher et al., 2001; Jackson, 2003; Weigl, 2005; International Union for Conservation of Nature, 2016; Nowak, 2018)

Dental Anatomy

Dental formula: I 3/1, C 0-1/0, PM 1-2/1-2, M 4/4

Macropods are often characterised by differences in their dentition and other anatomical adaptions to assist in processing species-specific diets. Intrinsically, macropods are often categorised as either a grazer, browser or a combination of both (mixed feeder) (Sanson, 1989; Clancy & Croft, 1991; Vogelnest & Portas, 2008; Arman & Prideaux, 2015; Fiani, 2015; Mitchell et al., 2018). The differences in dental morphology, and subsequent dietary classification [a detailed review of which can be found in Arman and Prideaux (2015)], relate to the size of the premolar, morphology of the molar crown, and molar occlusion (Figure 1.1a,b,c) (Sanson, 1989; Fiani, 2015). The 'grazers' have vestigial premolars, and molars with pronounced lophs (the elongated enamel ridges which run between the cusps) designed for effective grinding of a diet predominantly consisting of grasses (Figure 1.1a) (Vogelnest & Portas, 2008; Arman & Prideaux, 2015). The unique feature of the grazers is the sequential shedding and replacement of the molar teeth, known as molar progression (Vogelnest & Portas, 2008; Fiani, 2015). The 'browsers' do not shed molar teeth, and have large premolars for cutting, and molars that are well-adapted for crushing the thicker, fibrous vegetation that this group predominantly consumes (Figure 1.1b) (Jackson, 2003; Vogelnest & Portas, 2008; Arman & Prideaux, 2015; Campbell et al., 2016). As the name suggests, the 'mixed' feeders consume a diet that is rich in grass, but members of this group will also select browse (Vogelnest & Portas, 2008; Dawson, 2012). The dental anatomy of mixed feeders, with higher molar lophs and more even occlusal surfaces, allows for continuous molar wear throughout life; however, some species shed the premolar, which facilitates molar progression (Figure 1.1c) (Vogelnest & Portas, 2008). The diet provided to macropods in captivity should reflect the diet that they would consume in the wild, and should be designed to suit their dietary classification.

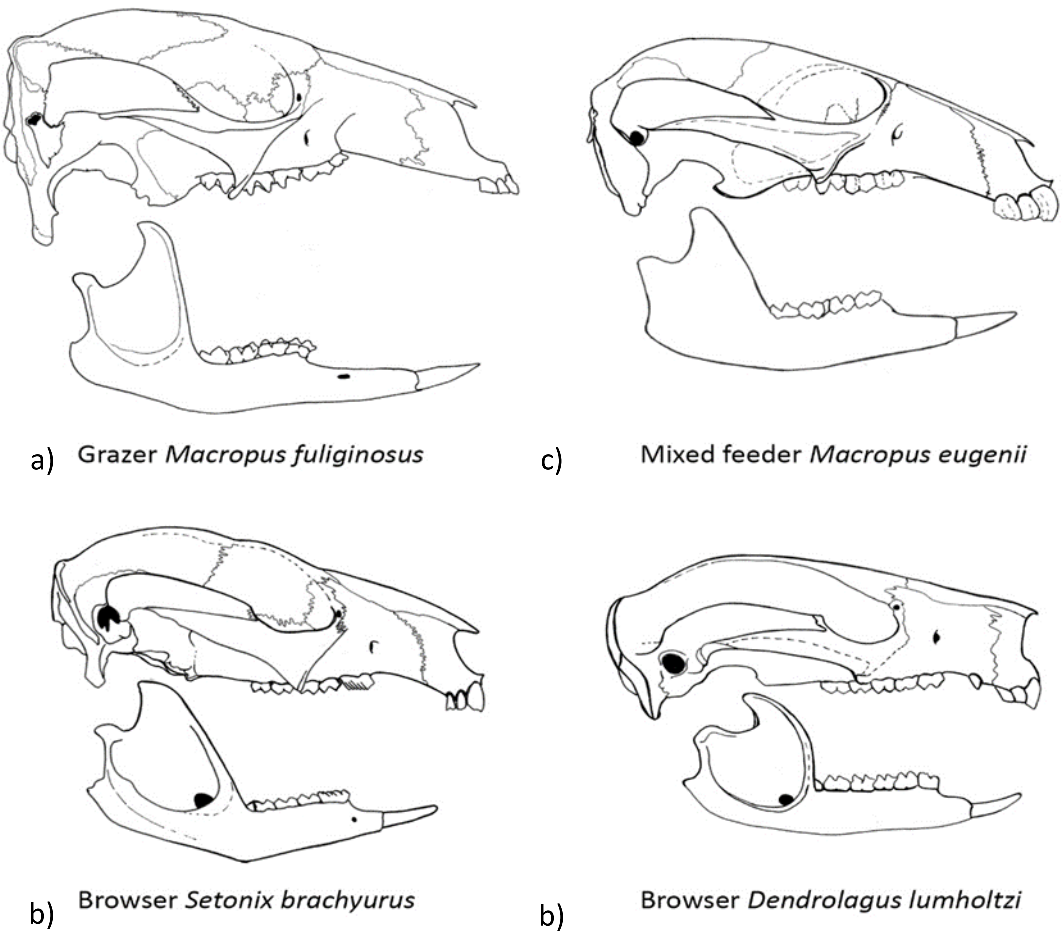

Figure 1.1: Visual representation of the differences in detention in macropod skulls and dentition for a) the grazer b) the browser and c) the mixed feeder categories adapted from Warburton (2009).

1.2.6 Macropods in captivity

Macropods have been housed in captivity since the late 1700's, where "The Wonderful Kangaroo from Botany Bay" (Jackson, 2003, p. 426) was displayed at the Lyceum in London, where the public could pay to observe this "amazing, beautiful and tame animal" from the southern hemisphere (Dawson, 1995, p. 4). Macropods are now housed in captivity to meet various aims, including: to conserve species through captive and reintroduction programs, for instance, the brush-tailed rock wallaby (*Petrogale penicillata*) (Schultz et al., 2006); to assist in education programs (Sherwen et al., 2015); for research (Wong et al., 2018), and for display in zoological institutions (Jackson, 2003; Hosey et al., 2013), with such display often also incorporating one or more of the other aforementioned aims. Macropods of many species are now housed in captivity in almost every continent around the world (Table 1.2) (Species360, 2018).

1.2.7 Geographic distribution of captive macropods

Global distribution of macropods housed in captivity now spans almost every continent (Table 1.2). As of 22nd October 2018, there are 7546 macropods from nine genera housed in captivity and registered with the Zoological Information Management System (ZIMS), a web-based database of zoo animal health and husbandry records often used in zoological institutions; only *Dorcopsulus* is not reportedly housed. This population of macropods is distributed across six regions, the two most populous of these regions (in terms of macropods housed) being Australasia (n = 2202) and Europe (n = 3530) (Species360, 2018) (ZIMS Data downloaded 22nd October 2018).

Table 1.2: Global distribution of captive macropods registered on the Zoological Information Management System (Species360, 2018).

Genus	**No. species**	**Region**	**No. institutions**	**No. in captivity**
Dendrolagus	4	Asia Australia Europe North America	48	114
Dorcopsis	1	Asia Europe	2	12
Lagorchestes	2	Australia	2	28
Macropus	11	Africa Asia Australia Europe North America South America	480	6381
Onychogalea	2	Australia	2	15
Petrogale	6	Asia Australia Europe North America	37	297
Setonix	1	Australia	13	58
Thylogale	4	Asia Australia Europe	24	149
Wallabia	1	Asia Australia Europe North America	49	492

1.2.8 Problems associated with maintaining macropods in captivity

Maintaining macropod populations in captivity is challenging, and requires multidisciplinary contributions and knowledge relating to housing and husbandry, feeding and nutrition, handling and transport, behavioural repertoire, breeding and the rearing of young, managing health, and minimising stress (Jackson, 2003; Morgan & Tromborg, 2007; Rees, 2011; Hosey et al., 2013). The captive environment may become a source of morbidity for macropods; therefore, to ensure healthy macropod populations, careful and continual monitoring of health, in addition to the environment, is essential.

Housing and husbandry

Housing and husbandry regimes help to minimise the risk of disease and stress in captive wild animals. Demands are placed on zoos to deliver housing that has several functions. Specifically housing should i) stimulate the animals mentally and physically, and enable them to form self-sustaining populations; ii) provide zoo visitors with a stimulating and educational experience; iii) be practical to maintain, so that keepers can effectively care for the animals (Hosey et al., 2013). Enclosures should meet the behavioural and physical needs of the individuals housed within them, at every stage of their life. Consideration should also be given to enclosure design, content, spatial requirements of the species, protection from extreme temperatures and weather, substrate, maintaining hygiene, and ensuring the compatibility of the animals housed together (Jackson, 2003; Hosey et al., 2013; Rendle et al., 2018). Housing wild animals in an artificial setting is difficult, especially when managing macropods in countries other than from where they originate. Zoos may attempt to replicate the natural ecological niche of species; however, managing macropod populations from differing ecological niches within one enclosure is demanding, such as red-necked wallabies (*Macropus rufogriseus*), that can be found in the alpine regions of Tasmania, and red kangaroos, which populate the arid, dry centre of Australia. Trends in enclosure type and design occur, and popularity of enclosure types change over time (Hosey et al., 2013); most often as the result of anecdotal rather than scientific evidence (Melfi, 2009). Recording changes in animal health and behaviour is an essential part of maintaining good husbandry.

Diet

Meeting the nutritional needs of captive macropods can be a challenge for zoos, and mirroring the natural diet should form the basis of diets in captivity. Zoos seek to provide a diet that is appropriate for the species, meets the nutritional needs of the individual, is consumed consistently, and stimulates natural feeding behaviours (Hosey et al., 2013; Johnson-Delaney, 2014). In addition, the diet should fit with the dentition and dietary classification for the species. The diet for captive macropods is often similar to those fed to ungulates and frequently comprises a fibrous pellet, supplemented with fresh vegetables and/or fruit, grass, hay and browse (Johnson-Delaney, 2014). Some items, such as browse and grass, may also occur naturally in the enclosure, and may constitute part of the captive diet.

Stress and disease in captive macropods

Wild animals housed in captivity may be adversely affected by environmental stimuli, resulting in stress (Morgan & Tromborg, 2007). Stressors in captivity often include those of a direct anthropogenic nature, such as the presence and proximity of zoo visitors, aversive sounds, smells, lighting, confinement, inappropriate diet/feeding regimes, abnormal social groupings and transportation (Broom, 2005; Morgan & Tromborg, 2007). Stress can lead to a range of issues which may have negative impacts on the viability of individuals and populations in captivity, such as reduced reproduction (Hing et al., 2014; Narayan & Hero, 2014; Hing et al., 2017). The effect of stress on the hypothalamic-pituitary-adrenal (HPA) axis has also been associated with immunosuppression, resulting in disease (Dohms & Metz, 1991; Blecha, 2000; Narayan, 2019). Macropods may be affected by a range of diseases including dermatological infections, neurological and pulmonary diseases and many others of an infectious or non-infectious nature. In captive macropods, the disease that is the most significant with respect to adverse impact is lumpy jaw (Jackson, 2003; Vogelnest & Portas, 2008; McLelland, 2019).

1.3 Lumpy jaw

Lumpy jaw was first reported in captive kangaroos at the Zoological Gardens of Copenhagen in 1890, when it was described as a disease reminiscent of calf diphtheria. The causative agent of calf diphtheria was known as *Fusobacterium necrophorum* or “Nekrosebaccillen” (Bang, 1890 cited in Burton, 1981, p. 1). This same agent was isolated from lesions from two kangaroos housed in Copenhagen. Shortly after this discovery, two further reports with similar clinical and bacterial presentations were observed in captive macropods in France (Nocard & Le Clainche, 1898) and Germany (Jensen, 1913). Since it was first documented, the disease has been reported at institutions throughout Australia, Europe, the United States of America and Asia (Burton, 1981; Ketz, 1997; Brookins et al., 2008; Vogelnest & Portas, 2008; Kido et al., 2013), and it is considered a leading cause of death in captive macropods (Jackson, 2003; Vogelnest & Portas, 2008).

1.3.1 Lumpy jaw in non-macropod species

Lumpy jaw has been reported in different types of animals including domestic, captive and wild populations (Table 1.3) (Oostman & Smego, 2005; Valour et al., 2014). The term ‘lumpy jaw’ is used to describe dental abnormalities comprising mandibular osteomyelitis, bony proliferation of the jaw bones, malocclusion and broken teeth, in species such as wild sheep (e.g. *Ovis dalli stonei*), with the conditions being similar to the disease observed in macropods (Hoefs & Bunch, 2001). Pathogenic agents are often involved; for example, *Actinomyces bovis*, giving rise to the disease name ‘actinomycosis’ (lumpy jaw) in cattle (Masand et al., 2015). Based on differences in clinical signs and pathogenic agents, it is unknown if the aetiological agents of lumpy jaw are the same across animal species, or even if they are the same in macropods (Hoefs & Bunch, 2001; Antiabong et al., 2013a; Agarwal & Chandra, 2014; Choudhary et al., 2016).

Table 1.3: Examples of non-macropod species for which lumpy jaw has been reported.

Species	Source
American bighorn sheep (*Ovis canadensis*)	Hoefs and Bunch (2001)
Blue duiker (*Cephalophus monticola fusicolor*)	Roeder et al. (1989)
Cape Mountain zebra (*Equus zebra zebra*)	Penzhorn (1984)
Dall's sheep (*Ovis dalli dalli*)	Hoefs and Bunch (2001)
Dik-dik (*Madoqua* sp.)	Wiggs and Lobprise (1994)
Domestic cat (*Felis catus*)	Soto et al. (2014)
Elk (*Cervus elaphus*)	Hoefs and Bunch (2001)
Klipspringer (*Oreotragus oreotragus*)	Wiggs and Lobprise (1994)
Malayan tapir (*Tapirus indicus*)	Da Silva et al. (2011)
Pronghorn antelope (*Antilocapra americana*)	Hoefs and Bunch (2001)
Rabbit (*Oryctolagus cuniculus*)	Harcourt-Brown (1995)
Stone's sheep (*Ovis dalli stonei*)	Hoefs and Bunch (2001)
Suni (*Neotragus moschatus*)	Wiggs and Lobprise (1994)
White-tailed deer (*Odocoileus virginianus seminlus*)	Chirino-Trejo et al. (2003); MacDonald and Labisky (2004)

1.3.2 Definition

Lumpy jaw is a complex syndrome which is characterised by proliferative osteomyelitis of the mandible and/or maxilla, associated with bacterial infection that causes soft tissue swelling within the dental arcade (Vogelnest & Portas, 2008; Vogelnest, 2015). It is considered a continuum of oral disease, whereby early stages of lumpy jaw could incorporate conditions from gingivitis through to periodontal disease (McLelland, 2019). The progressive nature of lumpy jaw was recently described by McLelland (2019), where the disease was defined as "multifactorial progressive inflammatory and necrotising polymicrobial disease associated with predominantly anaerobic opportunistic bacterial infection of the soft tissue and bony structures supporting the teeth, including gingivitis, periodontitis and mandibular/maxillary osteomyelitis". To reflect this broad definition, McLelland (2019) also proposed that the disease, as he defined, should be referred to as 'macropod progressive periodontal disease'. However, the range of pathological features required to make a diagnosis (discussed below) are often most easily observed in the mid to late stages of the disease, and 'lumpy jaw' is still the most commonly used term to refer to the disease, hence the use of this term in this study. The progressive nature of lumpy jaw makes it a condition which is challenging to define, and prior to this study, a case definition for the progressive stages of lumpy jaw had not been clearly defined in the literature.

The nomenclature of 'lumpy jaw'

Lumpy jaw is a disease of multifaceted aetiology which has led to a plethora of terms being used to describe the disease, with many terms used being associated with the bacterial species cultured by the investigating scientist (Burton, 1981). Historically, terms have included actinomycosis (Blair, 1916), streptothricosis (Blair, 1924), nocardiosis (Scott & Camb, 1925), oral necrobacillosis (Olivkov & Nossoova, 1940; Tsvetaeva, 1941; Burton, 1981; Borland et al., 2012); only the names kangaroo disease (Boyd, 1929), jaw disease (Noback, 1930; Beveridge, 1934) and lumpy jaw (Fox, 1923) are free of bacterial inferences. The expression 'lumpy jaw' is most commonly used by laymen, veterinarians and scientists to refer specifically to the disease in macropods (Burton, 1981; Vogelnest & Portas, 2008). The term is used to draw together the various aspects of the disease and the multiple clinical signs including the presence of bacteria, the associated infection of the soft tissue and bony components of head, neck and jaws (osteomyelitis). Given that several bacterial species may be cultured in reported cases of infections of the oral cavity in macropods considered to have lumpy jaw, the use of the inclusive, albeit colloquial term 'lumpy jaw' is used throughout this thesis.

1.3.3 Aetiology

Lumpy jaw is a disease considered to be of multifactorial aetiology. Several hypotheses on factors that may act as sufficient cause(s) for lumpy jaw have been proposed, including the presence of several bacterial agents (Samuel, 1983; Antiabong et al., 2013a), occurrence of periodontal disease, and the process of tooth eruption and molar progression (Clarke, 2003; Vogelnest & Portas, 2008; Vogelnest, 2015). Opportunistic pathogens gain entry through any breach in the hosts' natural defences, for example through trauma or disruption of the oral mucosa (Finnie, 1976;

Blyde, 1999; Vogelnest & Portas, 2008; Vogelnest, 2015). Infection and subsequent lumpy jaw can then ensue.

Bacteria

Several bacterial species, from nine genera (*Nocardia*, *Bacteroides, Actinomyces*, *Streptococcus*, *Staphylococcus, Fusobacterium, Porphyromonas*, *Pasteurella*, *Hemophilus*), have been cultured from the oral cavities of macropods affected with lumpy jaw (Table 1.4). Some of the key studies of bacteria associated with lumpy jaw were undertaken by Samuel (1982; 1983). These studies compared the oral flora of macropods with and without lumpy jaw, and noted that *Fusobacterium necrophorum* was absent in normal oral bacteria but was the most frequent bacterial isolate (81%) from lesions from macropods infected with "jaw disease" (Beveridge, 1934 cited in Samuel, 1983, p. 374). Similarly, Oliphant et al. (1984) carried out a detailed study of 27 wallabies with necrobacillosis of the face, legs and internal organs, and found that 69% of lesions examined contained *F. necrophorum*; the researchers concluded that this was "the main aetiological agent" responsible for disease outbreak (Oliphant et al., 1984, p. 383).

Although *F. necrophorum* is still considered to be the primary agent associated with lumpy jaw in macropods (Vogelnest & Portas, 2008; Antiabong et al., 2013a), several other bacterial species have also been isolated and there is disagreement in the literature as to the precise causative agent/s (Asperger et al., 2001). Several points remain to be clarified, including: whether the presence of *F. necrophorum* precedes the development of oral disease, or whether it is a secondary invader as a result of oral lesions; and whether *F. necrophorum* is a normal part of macropod oral flora or is introduced to the animal from the environment. Samuel (1982) studied the commensal oral flora of macropods and challenged the findings of previous researchers such as Irwin and Cameron (1962), Poelma (1964), Arundel et al. (1977), Miller et al. (1978) and Dent (1979), concluding that *F. necrophorum* is not present in the normal oral flora of macropods. However, in a later study which examined the bacterial flora of macropods affected with lumpy jaw, Samuel (1983) identified the involvement of both *F. necrophorum* and *Actinomyces.* Samuel (1983) postulated that the two organisms played an essential, synergistic role in the pathogenesis of lesions; supporting the earlier findings of Miller et al. (1978) that *Actinomyces* initiates the bone lesion and that *F. necrophorum* is an opportunistic secondary invader, consequently leading to the destruction of the bone and soft tissue. Burton (1981) argued that *Fusobacterium necrophorum* was 'independently responsible for the progression of infection', yet despite the high occurrence of lumpy jaw in captive macropods, experimental evidence demonstrated that macropods are no more susceptible to *F. necrophorum* than rabbits and mice when repeatedly challenged by subcutaneous injections of graded doses of *F. necrophorum*. This indicated that there are likely other predisposing factors necessary for the development of the disease (Smith et al., 1986; Vogelnest & Portas, 2008), and that it is likely that *F. necrophorum* does not act as a primary pathogen.

Irrespective of bacterial species, pathogens associated with lumpy jaw may invade the hosts' tissues when the oral mucosa is breached through trauma, abrasion, or through an associated debilitating condition such as periodontal disease (Finnie, 1976; Butler & Burton, 1980; Vogelnest & Portas, 2008).

Table 1.4: Bacteria cultured from macropods with lumpy jaw.

Bacterial genus	**Bacterial species (where known)**	**Reference**
Actinomyces	*Actinomyces* sp.; *A. pyogenes* *Actinobacillus* sp.	Blair (1916); Poelma (1964); Boever and Leathers (1973); Schneider et al. (1976); Schröder and Ippen (1976); Burton (1981); Samuel (1983); Hartley and Sanderson (2003)
Bacteroides	*B. melaninogenicus; B. oralis*; *B. bivius*; *B. disiens*; *B. fragilis*; *B. distasonis*; *B. thetaiotaomicron; B. vulgatus; Bacteroides rumenicola; B. ruminicola* subspecies *brevis; B. denticanoris*	Fox (1923); Watts and Mclean (1956); Keane et al. (1977); Taylor et al. (1978); Butler and Burton (1980); Oliphant et al. (1984); Antiabong et al. (2013a)
Fusobacterium	*F. nucleatum; F. necrophorum*	Bang (1890); Jensen (1913); Mouquet (1923); Burton (1981); Samuel (1983); Oliphant et al. (1984); Antiabong et al. (2013b)
Hemophilus	*H. hemolyticus*	Boever and Leathers (1973)
Nocardia	*Nocardia* spp.; *N. asteroids; N. macropodidarum*	Fox (1923); Le Souef and Seddon (1929); Tucker and Millar (1953); Eriksen (1964);

Bacterial genus	**Bacterial species (where known)**	**Reference**
Actinomyces	*Actinomyces* sp.; *A. pyogenes* *Actinobacillus* sp.	Blair (1916); Poelma (1964); Boever and Leathers (1973); Schneider et al. (1976); Schröder and Ippen (1976); Burton (1981); Samuel (1983); Hartley and Sanderson (2003)
Bacteroides	*B. melaninogenicus; B. oralis*; *B. bivius*; *B. disiens*; *B. fragilis*; *B. distasonis*; *B. thetaiotaomicron; B. vulgatus; Bacteroides rumenicola; B. ruminicola* subspecies *brevis; B. denticanoris*	Fox (1923); Watts and Mclean (1956); Keane et al. (1977); Taylor et al. (1978); Butler and Burton (1980); Oliphant et al. (1984); Antiabong et al. (2013a)
Fusobacterium	*F. nucleatum; F. necrophorum*	Bang (1890); Jensen (1913); Mouquet (1923); Burton (1981); Samuel (1983); Oliphant et al. (1984); Antiabong et al. (2013b)
Hemophilus	*H. hemolyticus*	Boever and Leathers (1973)
		Boever and Leathers (1973); Taylor et al. (1978)
Pasteurella	*P. multocida*	Young (1965)
Porphyromonas	*P. gingivalis*	Bird et al. (2002)
Staphylococcus	*Staphylococci* spp. *S. aureus*	Watts and Mclean (1956); Irwin and Cameron (1962); Burton (1981); Samuel (1983); Hartley and Sanderson (2003)
Streptococcus	*Streptococci* spp.	Irwin and Cameron (1962); Burton (1981); Samuel (1983); Oliphant et al. (1984); Hartley and Sanderson (2003)

Periodontal disease

Periodontal disease and lumpy jaw are not new phenomena in macropods, with evidence to suggest the presence of both of these conditions in mandibular fossils which are approximately 26,000 years old (Horton & Samuel, 1978). Periodontal disease is a chronic immuno-inflammatory condition of the periodontium (Pihlstrom et al., 2005; Oz & Puleo, 2011). In its mildest form, gingivitis, the bacterial biofilm (dental plaque) that accumulates on teeth adjacent to the gingiva, does not affect the supportive structures of the dentition (Pihlstrom et al., 2005; Oz & Puleo, 2011). However, periodontal disease is progressive, and initiated by the biofilm containing periodontal pathogens, including those associated with lumpy jaw. Periodontal disease can result in loss of gingival tissue, ligaments and supporting alveolar bone (Oz & Puleo, 2011; Antiabong et al., 2013b). It has been widely reported that the presence of bacteria, and development of dental plaque and calculus, create an environment where oral diseases, including periodontal disease, can occur in zoo-housed macropods (Finnie, 1976; Miller et al., 1978; Burton, 1981; Bird et al., 2002; Clarke, 2003; Bakal-Weiss et al., 2010; Antiabong et al., 2013a). Consequently, periodontal disease is often considered as a potential precursor to lumpy jaw (Burton, 1981; Clarke, 2003; Borland et al., 2012; Antiabong et al., 2013b).

Molar progression

Lumpy jaw in macropods is considered to be associated with the occurrence of molar progression (Clarke, 2003; Vogelnest & Portas, 2008), a process which affects all grazing macropods and some mixed browser-grazer feeders (Kirkpatrick, 1964; 1965; 1970; Clarke, 2003; Lentle et al., 2003; Vogelnest & Portas, 2008; Smith, 2009; Vogelnest, 2015). Molar progression refers to the rostral movement of molar teeth along the interdental space, where worn premolars and molars are sequentially shed and replaced as the macropod ages (Kirkpatrick, 1964; 1965; 1970; Clarke, 2003; Lentle et al., 2003; Vogelnest & Portas, 2008; Smith, 2009; Vogelnest, 2015). The occurrence of molar progression is thought to have an associative role with lumpy jaw due to two hypothesised factors: i) it creates a breach in the mucosa facilitating entry for pathogenic bacteria (Finnie, 1976; Arundel et al., 1977); and ii) 'post-functional' molar teeth create a trap for particles of food and pathogens, resulting in opportunistic infection (Miller & Beighton, 1979). However, the rate at which molar progression occurs is influenced by several factors, including macropod diet and the subsequent wear on dentition (Burton, 1981; Lentle et al., 2003), as well as species and sex (Kirkpatrick, 1970; Newsome et al., 1977). Sexual dimorphism in dental development, and subsequent molar progression, have been noted in few species, and this aspect could be species-specific (Kirkpatrick, 1970; Newsome et al., 1977). However, as lumpy jaw is a condition highly correlated with macropod age, the role that molar progression plays in the development of lumpy jaw will be influenced by the length of time the individual has been exposed to factors that influence the process, and these factors warrant further investigation.

1.3.4 Clinical signs

Lumpy jaw may present with a range of clinical signs depending on the location, severity, and stage of the disease process at the point of detection (Vogelnest & Portas, 2008). The most common site of infection is the periodontal region, with clinical presentation of the disease, as the name suggests, being swelling of the mandibular or maxillary region (Burton, 1981; Vogelnest & Portas, 2008) (Figure 1.2). Alveolar osteomyelitis (Brookins et al., 2008; Vogelnest & Portas, 2008), necrosis of gingival mucosa (Antiabong et al., 2013a), hyperptyalism (Vogelnest & Portas, 2008), halitosis, and the visible presence of an infected tooth which may include discharging abscesses (Finnie, 1976; Butler & Burton, 1980; Hartley & Sanderson, 2003; Vogelnest & Portas, 2008) are commonly presented. Unilateral ocular or nasal discharge (Fox, 1923; Mouquet, 1923; Vogelnest & Portas, 2008), blepharospasm (Vogelnest & Portas, 2008), respiratory distress, anorexia, and dysphagia have also been observed (Fox, 1923; Keane et al., 1977; Burton, 1981; Vogelnest & Portas, 2008; Vogelnest, 2015). Clinical signs of systemic illness, such as progressive emaciation, depression and lethargy (Vogelnest & Portas, 2008; Staker, 2014), may be the result of haematogenous spread of infection (Keane et al., 1977; Vogelnest & Portas, 2008), and also the production of a potent leukotoxin from *F. necrophorum* (Bennett et al., 2009). This action could lead to subsequent infections in the liver and stomach, as observed in several studies (Finnie, 1976; Schröder & Ippen, 1976; Arundel et al., 1977; Clarke, 2003; Vogelnest & Portas, 2008).

Figure 1.2: Swelling of the mandibular region (encircled) associated with clinical signs of lumpy jaw in a red kangaroo (*Macropus rufus*) (Images courtesy of Perth Zoo).

1.3.5 Gross pathology

The lesions associated with lumpy jaw are notoriously both necrotising and purulent, causing inflammation of soft tissue and severe necrosis of the jaw bone (Figure 1.3) (Clarke, 2003; Vogelnest & Portas, 2008). Early lesions have a central necrotic core surrounded by an area of oedematous soft tissue (Miller et al., 1978; Vogelnest & Portas, 2008), whilst in chronic lesions, extensive necrosis and lysis of the jaw bone will be observed (Miller & Beighton, 1979; Clarke, 2003; Vogelnest & Portas, 2008). This necrotic element of the disease frequently leads to tooth loss and pathological fractures of the mandible can occur (Clarke, 2003). As a result of aspirated infected material (saliva or purulent material), secondary infections, such as pneumonia, and abscesses may also occur in the lungs (Fox, 1923; Clarke, 2003). Necrotic abscesses are a common occurrence in visceral organs (Vogelnest, 2015) and have also been reported in the hind legs and tail (Finnie, 1976).

Figure 1.3: Lumpy jaw in a red-necked wallaby (*Macropus rufogriseus*). The image demonstrates calculus deposits and advanced bone loss exposing the root and furcation (Clarke, 2003).

1.3.6 Diagnosis of lumpy jaw

Diagnosis of lumpy jaw is based upon the observation of clinical signs, bacterial culture, haematology, and a detailed examination of the oral cavity to identify affected teeth and the presence of lesions (Lewis et al., 1989; Clarke, 2003; Vogelnest & Portas, 2008; Vogelnest, 2015). Radiography is used to determine the extent of involvement of the teeth and bones (Vogelnest & Portas, 2008), and rarely, computed tomography (CT) may be utilised to reveal sequestra formation not visible through radiography (Kane et al., 2017). Haematology may reveal abnormal neutrophil morphology, monocytosis, increased platelet counts, and deviations in red blood cell indices (Burton, 1981; Hawkey et al., 1982). Biochemical anomalies may include elevated creatine kinase (CK), indicative of inflammation or damage to muscles, and elevated aspartate aminotransferase (AST) if there is involvement of the liver; although this enzyme may also indicate tissue inflammation (Hawkey et al., 1982; Vogelnest & Portas, 2008). Hawkey et al. (1982) also found raised fibrinogen levels in all red-necked wallabies with confirmed lumpy jaw, however, elevated fibrinogen levels are also an indicator of non-specific inflammation, therefore may not be a useful tool for diagnosis of lumpy jaw.

Lumpy jaw is difficult to detect during the primary stages of development, and screening for the disease could therefore assist with early diagnoses and the provision of a suitable and effective treatment regime. However, the risks associated with capture and subsequent anaesthesia required to perform a clinical examination need to be balanced against the benefits associated with screening. Reliance on keeper observation of subtle changes in behaviour and feeding may be critical for the early detection, treatment and eventual outcome of lumpy jaw in captive macropods.

1.3.7 Treatment

Treatment for lumpy jaw is challenging, and a range of approaches, incorporating antibiotic therapies and a variety of surgical techniques have been trialled, with variable success (Wilson et al., 1980; Burton, 1981; Blyde, 1993; Hartley & Sanderson, 2003; Bakal-Weiss et al., 2010; Kilgallon et al., 2010; Kane et al., 2017). The location and extent of the lesions may determine the treatment that is provided (Lewis et al., 1989), and the level of success of any treatment may also be affected by the extent of post-operative care (Hartley & Sanderson, 2003). There are no known treatments that elicit a complete cure (Sotohira et al., 2017b), and attempts to find the most effective treatment approach are ongoing.

Antibiotics

Antibiotics are frequently used in the treatment of lumpy jaw in macropods, with several studies advocating the use of specific antibiotics, dose rates and delivery methods (Chapter 5, Table 5.1, p. 146). Vigorous antibiotic therapy can be offered in the initial stages of treatment of lumpy jaw (Kilgallon et al., 2010), however, the efficacy of an antibiotic product will be determined in part by the sensitivity of the organism (bacteria) to the antibiotic, and the ability of the antibiotic to penetrate the site of the infection (Butler & Burton, 1980). Antibiotics may be selected based on the findings of bacterial culture, however, several products may be trialled before a clinical resolution is achieved (Butler & Burton, 1980). Antibiotics may be delivered orally, parentally or topically; this includes the use of antibiotic rinses. The use of antibiotic rinses during irrigation of the oral cavity, or the use of a combination of antibiotic medication together with antiseptic rinses, such as povidone-iodine (Betadine®, Sanofi-Aventis, Virginia, Queensland, Australia), have also been used to manage the disease (Fagan et al., 2005; Kane et al., 2017). However, this therapy requires repetition and often culminates in the need for additional treatments (Fagan et al., 2005). Treatment with antibiotics has known disadvantages, including the delivery of suboptimal concentrations of the product to the infected tissues, which can be due to difficulties associated with repeated handling for the administration of antibiotics (Hartley & Sanderson, 2003). The use of long-acting (LA) antibiotics such as LA-oxytetracycline mitigates the need for repeated handling, however, more research is required into the pharmacokinetics of LA-oxytetracycline and other antibiotics used in macropods (McLelland et al., 2011). Parenteral treatment is also often preferred given the foregut fermenting digestive strategy of macropods, and the potential additional stressors associated with capture and restraint required to deliver therapy in species known to be affected by exertional myopathy (Jackson, 2003; McMahon et al., 2013; Green-Barber et al., 2018). Furthermore, the prolonged use of particular antibiotics, including oxytetracycline, can cause diarrhoea; which presents further health challenges for macropods already affected by the debilitating effects of lumpy jaw. To achieve the best outcome for the macropod, antibiotics are often used in combination with surgical intervention. Surgical intervention is also often advocated after a period of unsuccessful antibiotic therapy (Fagan et al., 2005; Vogelnest & Portas, 2008).

Surgery

Initial treatment commonly involves the extraction of infected teeth and curettage of necrotic material (Lewis et al., 1989; Bodley et al., 2005; Vogelnest & Portas, 2008). Successful surgical treatment for lumpy jaw has also included endodontic therapy and apicoectomy (Kilgallon et al., 2010), and the use of antibiotic impregnated polymethylmethacrylate (AIPMMA) beads implanted at the site of infection (Hartley & Sanderson, 2003; Kane et al., 2017). The beads deliver antibiotics directly to the infected site, thus reducing the need for repeated handling. However, post-surgical care requires particular consideration, specifically the use of analgesia (Kirkwood et al., 1988; Bakal-Weiss et al., 2010), as well as the management of post-surgical complications, including wound infections. Oral varnishes may be applied post-surgery to minimise infection, and have also been used as a stand-alone method to treat lumpy jaw infections (Bakal-Weiss et al., 2010). The direct application of a disinfectant varnish, such as chlorhexidine, to the affected teeth and gingivae, has the advantage of inhibiting the development of gingivitis or early periodontal dental disease due the varnish's antimicrobial properties (Bakal-Weiss et al., 2010). However, once dental disease is present, varnish alone will not be effective, and a combination of varnishes, systemic antibiotic therapy and surgical intervention is typically required.

Unfortunately, lumpy jaw is often not identified until it has reached an advanced stage, where treatment is not considered effective, in which case euthanasia is the most humane option (Vogelnest & Portas, 2008; Vogelnest, 2015). Detailed studies are necessary to assess the outcome of treatments and to draw conclusions as to the efficacy of specific therapies.

Prophylaxis

Given treatment for lumpy jaw can be long, protracted and not always efficacious preventative measures should be explored. Preventive vaccination programs have been trialled in captive macropods, but to date have had limited success (Smith et al., 1985; Smith et al., 1986; Phillips et al., 2012). Vaccines targeted towards *F. necrophorum* failed to induce resistance in captive red-necked wallabies (Smith et al., 1986). Trials targeting other bacterial species reported in cases of lumpy jaw, *Dichelobacter nodosus*, initially appeared protective, but generally were reported to be inefficient in reducing the number of cases of lumpy jaw (Smith et al., 1986; Blanden et al., 1987; Phillips et al., 2012). Therefore, to date an effective preventative treatment is unavailable.

1.3.8 Geographic and host distribution

Lumpy jaw has been reported from 12 countries and in several macropod species held in captivity worldwide (Table 1.5). The disease has been observed repeatedly in captive populations, with the eastern grey kangaroo (*Macropus giganteus*), red kangaroo and red-necked wallaby over-represented in these studies, as demonstrated in Table 1.5. However, this may be indicative of the popularity of these species in captivity, rather than a species susceptibility to lumpy jaw. Although it is interesting to note the locations of studies, these figures do not provide information of all the macropod species kept in captivity globally; as not all institutions, and their macropods, are registered with the global database, Zoological Information Management System (ZIMS). Additionally, these figures do not truly represent the extent of the disease in captivity or in the wild, as knowledge of the disease prevalence is often limited to institutional or case studies of lumpy jaw.

There are few studies of lumpy jaw in wild macropods and many are based on opportunistic, rather than systematic, studies of the disease (e.g. Borland et al., 2006; 2012). For example, many reports of the disease in wild macropods are based on individual cases (Tomlinson & Gooding, 1954; Arundel et al., 1977). The large number of cases reported in the Borland et al. (2012) study provided evidence of widespread lumpy jaw in wild individuals; however, these observed cases were associated with periods of extreme environmental stress (drought), potentially leading to overcrowding, limited essential resources such as food and water, and substantial faecal contamination of pastureland. With so few studies conducted on this disease in wild macropods, we know very little about the nature of lumpy jaw in free-living animals (Arundel et al., 1977; Borland et al., 2012), and very little about its prevalence in the wild (Borland et al., 2012). Extrapolation from studies of lumpy jaw in captive populations permits aetiological and epidemiological investigations of lumpy jaw to be performed, and may assist in developing an understanding of the disease in wild populations.

Table 1.5: Reports of lumpy jaw in both captive (c) and wild (w) macropods.

Genus	**Species common name**	**Location (c) (w)**	**Reference**
Macropus	Agile wallaby	Australia (c)	Burton (1981)
	Common wallaroo/euro	Australia (c/w)	Vogelnest and Portas (2008)
	Eastern grey kangaroo	Australia (c)	Burton (1981)
		Australia (w)	Borland et al. (2012)
		Australia (c/w)	Vogelnest and Portas (2008)
		Israel (c)	Bakal-Weiss et al. (2010)
		USA (c)	Boever and Leathers (1973)
		Germany (c)	Kronberger and Schüppel (1976); Schneider et al. (1976);Lindau (1964)
		Croatia (c)	Huber et al. (1976)
		Japan (c)	Kido et al. (2018)
	Parma wallaby	Australia (c)	Burton (1981); Vogelnest and Portas (2008)
		New Zealand (c)	Meadows (1981)
	Red kangaroo	Australia (w)	Tomlinson and Gooding (1954)
		Australia (c/w)	Vogelnest and Portas (2008)
		Australia (c)	Burton (1981)
		USA (c)	Boever and Leathers (1973); Brookins et al. (2008)
		South Africa (c)	Young (1965)
	Red-necked wallaby/Bennett's wallaby	UK (c)	Oliphant et al. (1984); Canfield and Cunningham (1993); Hartley and Sanderson (2003)
		Germany (c)	Asperger et al. (2001)
		Australia (c/w)	Vogelnest and Portas (2008)
		Denmark (c)	Bertelsen et al. (2012)
		New Zealand (c)	Meadows (1981)
		Israel (c)	Bakal-Weiss et al. (2010)
	Tammar wallaby	Australia (c)	Barker (1971)
		Australia (w)	Arundel et al. (1977)
		New Zealand (c)	Meadows (1981)
		Hungary (c)	Sós et al. (2012)
		UK (c)	Blanden et al. (1987)
	Western grey kangaroo	Australia (c)	Vogelnest and Portas (2008)
	Whiptail wallaby	Australia (c/w)	Vogelnest and Portas (2008)
Onychogalea	Bridled nail-tail wallaby	Australia (c/w)	Vogelnest and Portas (2008)
Petrogale	Yellow-footed rock wallaby	Australia (c)	Vogelnest and Portas (2008); Antiabong et al. (2013a)
Thylogale	Red-necked pademelon	Australia (c/w)	Vogelnest and Portas (2008)
Wallabia	Swamp wallaby	Australia (w)	Arundel et al. (1977)
		Australia (c/w)	Vogelnest and Portas (2008)
		Australia (c)	Burton (1981)
		Japan (c)	Kido et al. (2013)
Unspecified	Unspecified	Denmark (c)	Bang (1890); Eriksen (1964)
		Germany (c)	Jensen (1913); Ketz (1997)
		France (c)	Nocard and Le Clainche (1898)
		Australia (c/w)	Samuel (1983)

1.4 Epidemiology of lumpy jaw

Epidemiology is the study of trends and patterns of disease within populations, and may be used to solve disease-related problems. Epidemiological investigations may also form the benchmark for the development of recommendations to prevent the occurrence and spread of disease (Thrusfield & Christley, 2018), such as lumpy jaw. The characteristic endemicity and cause(s) of lumpy jaw within captive macropod populations are largely unknown, and crucially, are unmeasured; therefore, an epidemiological investigation of lumpy jaw, including the risk factors associated with its occurrence, is important to enable the development of recommendations for the management and control of this disease in captive populations.

1.4.1 Prevalence

Reported prevalence of lumpy jaw in macropods is typically inconsistent, with a wide range of figures presented for the disease within captive and wild populations (Table 1.6). Prevalence (P) is defined as the proportion of cases of disease in a given population at a designated time (Thrusfield & Christley, 2018). When prevalence is reported in the literature, it is often based on the result of necropsy findings. In the captive setting, prevalence figures are often calculated from necropsy reports, while in the wild they are frequently the result of opportunistic findings. Therefore, the prevalence figures reported are often based on a skewed (non-living) population; they are not representative for captive individuals that received successful treatment for lumpy jaw, nor for wild macropods where the outcome is often unknown (Wobester, 2006). Table 1.6 provides a range of known prevalence of lumpy jaw, calculated from necropsy reports and veterinary diagnoses during opportunistic findings, from both captive and wild macropod species.

Prevalence in the wild

Knowledge of prevalence of disease in wild populations provides a useful benchmark for disease parameters for captive populations; however, few substantiated records of disease occurrence exist. Lumpy jaw has a long history of occurrence in wild macropod populations. The earliest known prevalence is reported in the now-extinct *Macropus titan* from the Pleistocene era; at 1.6% (95% CI: 0.04 - 8.66) (Horton &

Samuel, 1978), but the disease is generally considered to be present in low levels in extant wild macropod populations (Wallach, 1971; Vogelnest & Portas, 2008). Nevertheless, as Table 1.6 demonstrates, prevalence in wild macropods can vary considerably; although with respect to these figures, measuring disease in wild animals is challenging as clinical examination without chemical immobilisation is often not possible, which may lead to distorted disease detection rates (Wobester, 2006). Chapter 6 contains a more detailed explanation of these challenges.

Prevalence in captivity

Lumpy jaw is one of the most frequently observed diseases in captive macropods, and is thought to affect all species (Jackson, 2003; Vogelnest & Portas, 2008). However, there is great variation in prevalence of lumpy jaw between species (Table 1.6). Prevalence reports are often based on results from a single species or institution, and therefore provide a biased indication of disease presence in captivity. Institutional differences in management practices may contribute to differences in prevalence, and also the geographic location of the zoo may be a contributory factor (Kido et al., 2013). In Japan, Kido et al. (2013) found a significantly higher prevalence of the disease (P = 40.7%, 95% CI: 27.57 – 55.97) for the swamp wallaby (*Wallabia bicolor*), compared to that found in Australia (P = 9.5%, 95% CI: 4.20 - 17.91) (Vogelnest & Portas, 2008). Contributing factors such as differences in environmental conditions between the two countries, diet, and enclosure type and size, may be hypothesised as potential influences on prevalence rates; hypotheses that have yet to be investigated.

For some species, the data does not provide a true indication of prevalence of lumpy jaw in the wild or captivity. Some species have been reported as having 0% prevalence (see Table 1.6), however sample sizes of animals in captivity are generally small, and therefore it is difficult to draw any meaningful conclusions. For example, prevalence of lumpy jaw reported for quokka (*Setonix brachyurus*) in studies by Vogelnest and Portas (2008), were based on n = 1. Therefore, a widespread study using sample sizes based on power analysis would be beneficial to determine prevalence of lumpy jaw in captive populations and any particular species susceptibility.

Table 1.6: Reported prevalence (P), 95% confidence intervals (CI) and sample size (n) for lumpy jaw in captive (c) and wild (w) macropods (ud – undetermined).

Genus	**Species common name**	**P % (x/n)**	**95% CI**	**Status**	**Reference**
Dendrolagus	Doria's tree kangaroo	100 (1/1)	2.5 - 100	c	Butler and Burton (1980)
	Matschie's tree kangaroo	60.0 (3/5)	14.7 - 94.7	c	Butler and Burton (1980)
Dorcopsis	Brown dorcopsis	7.7 (1/13)	0.2 – 36.0	c	Butler and Burton (1980)
Lagorchestes	Rufous hare-wallaby	0.0 (0/17)	0.0 - 19.5	c/w	Vogelnest and Portas (2008)
Macropus	Agile wallaby	29.0 (18/62)	18.2 – 41.9	c	Butler and Burton (1980)
	Black-striped wallaby	50.0 (4/8)	15.7 – 84.3	c	Butler and Burton (1980)
	Common wallaroo	17.5 (11/63)	9.1 - 29.1	c/w	Vogelnest and Portas (2008)
		21.4 (3/14)	4.7 – 50.8	c	Butler and Burton (1980)
	Eastern grey kangaroo	5.1 (13/256)	2.7 - 8.5	c/w	Vogelnest and Portas (2008)
		39.5 (17/43)	25.0 - 55.6	c	Butler and Burton (1980)
		39.4 (13/33)	22.9 - 57.9	c	Bakal-Weiss et al. (2010)
		53.9 (48/89)	43.0 - 64.6	w	Borland et al. (2012)
	Parma wallaby	14.3 (2/14)	1.8 - 42.8	c/w	Vogelnest and Portas (2008)
		37.5 (12/32)	21.1 – 56.3	c	Butler and Burton (1980)
	Red kangaroo	29.6 (16/54)	18.0 - 43.6	c/w	Vogelnest and Portas (2008)
		58.3 (14/24)	36.6 - 77.9	c	Butler and Burton (1980)
		ud	ud	w	Tomlinson and Gooding (1954)
	Red-necked wallaby	9.3 (9/97)	4.3 - 16.9	c/w	Vogelnest and Portas (2008)

Genus	Species common name	P % (x/n)	95% CI	Status	Reference
	Red-necked wallaby cont.	33.3 (3/9)	7.5 – 70.1	c	Butler and Burton (1980)
	Tammar wallaby	20.0 (2/10)	2.5 – 55.6	c	Butler and Burton (1980)
		0.0 (0/4)	0.0 - 60.2	c/w	Vogelnest and Portas (2008)
		ud	ud	w	Arundel et al. (1977)
	Western grey kangaroo	2.6 (1/39)	0.1 - 13.5	c/w	Vogelnest and Portas (2008)
		0.0 (0/18)	0.0 – 18.5	c	Butler and Burton (1980)
	Whiptail wallaby	6.8 (4/59)	1.9 - 16.5	c/w	Vogelnest and Portas (2008)
		0.0 (0/3)	0.0 – 70.8	c	Butler and Burton (1980)
Onychogalea	Bridled nail-tail wallaby	15.5 (9/58)	7.3 - 27.4	c/w	Vogelnest and Portas (2008)
	Northern nail-tail wallaby	100 (1/1)	2.5 - 100	c	Butler and Burton (1980)
Petrogale	Brush-tailed rock wallaby	0.0 (0/1)	0.0 – 97.5	c	Butler and Burton (1980)
	Yellow-footed rock-wallaby	13.0 (3/23)	2.8 - 33.6	c/w	Vogelnest and Portas (2008)
Setonix	Quokka	0.0 (0/1)	0.0 - 97.5	c/w	Vogelnest and Portas (2008)
		14.3 (1/7)	0.4 – 57.9	c	Butler and Burton (1980)
Thylogale	Red-necked pademelon	0.0 (0/9)	0.0 - 33.6	c/w	Vogelnest and Portas (2008)
	Tasmanian pademelon	6.7 (1/15)	0.2 – 31.9	c	Butler and Burton (1980)
Wallabia	Swamp wallaby	9.5 (8/84)	4.2 - 17.9	c/w	Vogelnest and Portas (2008)
		40.7 (22/54)	27.6 - 55.0	c	Kido et al. (2013)
		50.0 (8/16)	24.7 – 75.3	c	Butler and Burton (1980)
		ud	ud	w	Arundel et al. (1977)

1.4.2 Risk factors and measuring risk

Although prevalence is a useful measure of proportion of disease, incidence rates would provide additional information and offer an indication of the level of risk to the population. There are no known reports of incidence rates, or studies that specifically measure risk factors for the development of clinical lumpy jaw. This information would be beneficial for reducing cases of disease in captivity, and ultimately beneficial to the zoo community worldwide, so that processes could be implemented to prevent this refractory disease.

Postulated risk factors for lumpy jaw include the presence of infectious agents (Burton, 1981; Samuel & Fowler, 1981; Vogelnest, 2015), factors associated with the host (Kido et al., 2013; Kido et al., 2018) and the environment (Finnie, 1976; Burton, 1981; Butler, 1981; Ketz, 1997; Borland et al., 2012). However, some studies have also implicated periods of acute stress in outbreaks of the disease (Finnie, 1976; Vogelnest & Portas, 2008; Borland et al., 2012). The congregation and high-density housing of macropods in captivity can create an environment where lumpy jaw becomes a commonly observed condition (Butler, 1981; Vogelnest & Portas, 2008). In the wild, lumpy jaw is considered uncommon (Wallach, 1971; Vogelnest & Portas, 2008; Borland et al., 2012), appearing during periods of extreme and adverse phenomena, for example, drought or flood (Butler, 1981; Borland et al., 2012). In captivity, lumpy jaw is observed frequently, suggesting factors associated with captive management are sub-optimal and predispose animals to the condition. Figure 1.4 explores the causal relationships for lumpy jaw in captive macropods.

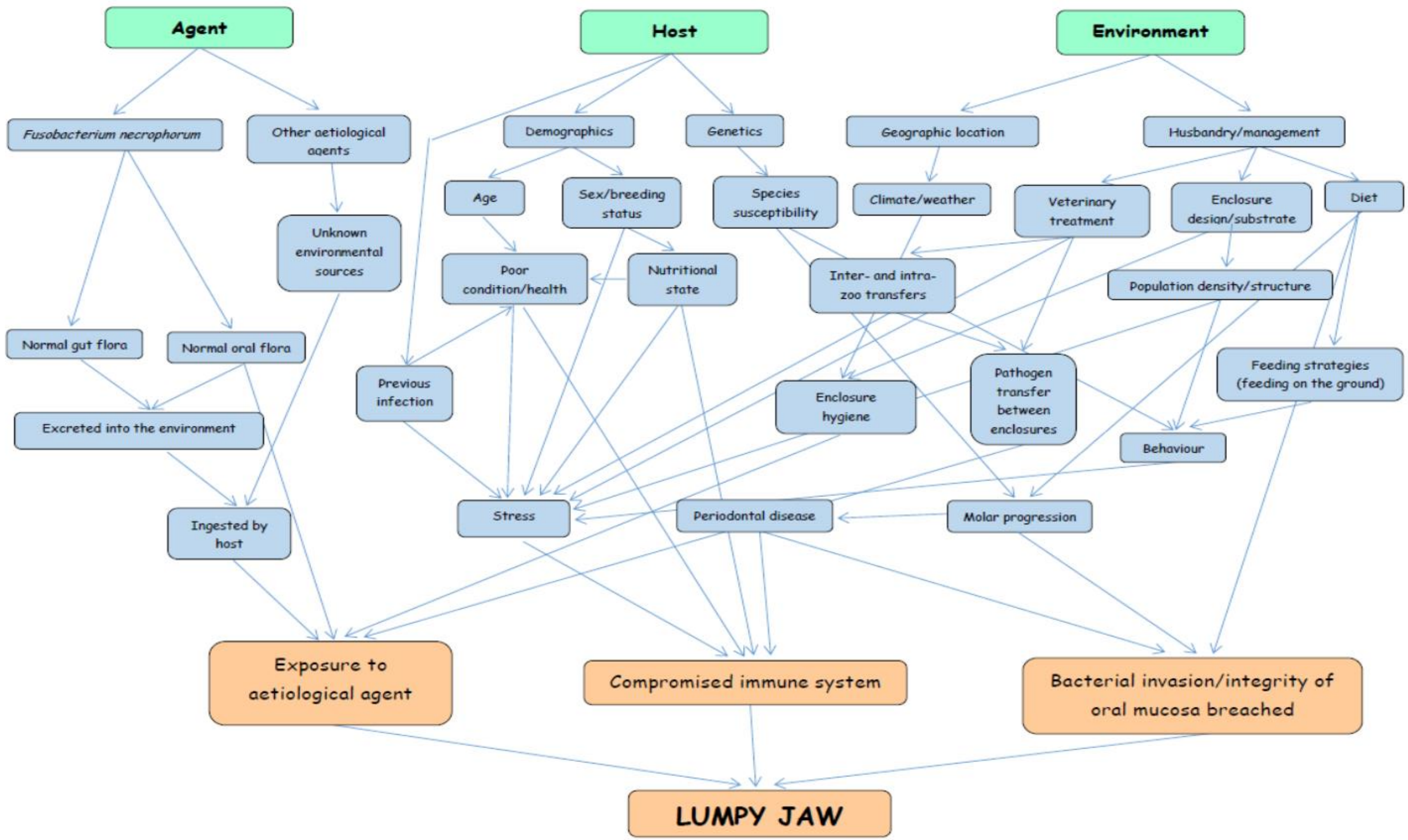

Figure 1.4: Causal web demonstrating the potential risk factors involved with the development of lumpy jaw in captive macropods.

1.4.3 Agent factors

Lumpy jaw is frequently associated with factors in the environment, including the presence of pathogenic bacteria. The synergism between *F. necrophorum* and other bacteria is not limited to lumpy jaw in macropods, it has also been reported as a highly significant component of diseases such as footrot in sheep and cattle (Roberts & Egerton, 1969; Emery et al., 1985; Bennett et al., 2009). Footrot serves as an important comparison to lumpy jaw, as it is also a disease associated with quality of the environment (Whittington, 1995; Whittier & Umberger, 2009). A recent study identified that a primary bacterium, *Dichelobacter nodosus*, appeared to be responsible for the onset of footrot (interdigital dermatitis), and *F. necrophorum* was only associated once severe footrot had taken hold (Witcomb et al., 2014); suggesting that *F. necrophorum* plays a more opportunistic than synergistic role. Yet a vaccine against *D. nodosus* in tammar wallabies, as discussed earlier, was potentially protective; by providing immunity against other bacteria that may be working synergistically with *F.* necrophorum (Blanden et al., 1987). Evidence of transferal of *F. necrophorum* (via 'an un-described pathway') from the environment to the animal (sheep), and/or vice versa, has been suggested by Bennett et al. (2009); with presence of *F. necrophorum* being identified on oral swabs taken from sheep with footrot. Understanding the route of transmission is important for the control and management of disease. Footrot is controlled through good management practices, which may include managing pasture hygiene, the removal of infected animals, and reduced stocking density (Winter et al., 2015; Dickins et al., 2016; Witt & Green, 2018). Burton (1981) and Ketz (1997) have argued that practices similar to these should also be adopted to reduce cases of lumpy jaw in captive macropods.

Environmental contamination with pathogen-infected faeces or discharges is generally believed to be a significant factor in the development of lumpy jaw (Fox, 1923; Beveridge, 1934; Burton, 1981; Vogelnest & Portas, 2008). It was postulated by Barker et al. (1963) that the source of bacterial agents in the oral cavity could be the upper gastrointestinal tract, due to the regurgitation activity known to occur in some marsupials, known as merycism. In addition, consumption of faecal matter whilst grazing is also postulated as a possible route of transmission of bacteria involved in the disease (Vogelnest & Portas, 2008; Borland et al., 2012). The ingestion of faecal matter containing *F. necrophorum* is highly likely when macropods congregate in large numbers, due either to environmental pressures in the wild, or through captivity; particularly as *F. necrophorum* has been suggested as being a normal inhabitant of the gastrointestinal tract (Nagaraja et al., 2005). *Fusobacterium necrophorum* is a strict anaerobe with a high level of sensitivity to oxygen, but appears capable of survival in the environment for extended periods (Ketz, 1997; Nagaraja et al., 2005; Takahashi, 2005). It is suggested that this survival is due to the protection of *F. necrophorum* by other bacteria, usually aerobes (Burton, 1981). The contribution of environmental contamination with faeces to the development of lumpy jaw is further supported by the results from a feed trial in which Burton (1981) contaminated the feed of a number of wallabies with *F. necrophorum*. Burton (1981) discovered that *F. necrophorum* was able to survive on fresh food items throughout the night when the weather was dry and mild. This bacterium was also found to survive on pelleted food in laboratory conditions at 25°C, although the precise environmental requirements for the survival of *F. necrophorum* are yet to be established. However, to reduce the risk of bacterial presence, food preparation should also include appropriate hygiene measures (Rees, 2011). Current evidence of a seasonal environmental influence on bacterial survival is conflicting. The survival of *F. necrophorum* may not necessarily be dependent on warm and dry conditions as suggested by Burton's in vitro study (1981), as a significant outbreak resulted in the death of 200 wallabies during an exceptionally cold season at a British zoo (Oliphant et al., 1984). This, coupled with the Burton (1981) report on higher occurrence at Melbourne Zoo during the Australian autumn/winter, demonstrates this disease occurs across regions and seasons. Development of the disease may be a result of environmental stress resulting in immunosuppression, rather than survival of the organism in the environment, and other factors, likely multiple, must be considered in regard to the aetiology of lumpy jaw.

1.4.4 Environmental factors

As previously discussed, there are a range of risk factors for lumpy jaw in the captive environment, some of which may be managed by zoo personnel (e.g. environmental control of pathogenic bacteria and biosecurity), whilst other risk factors are out of institutional control. Ensuring a healthy and clean environment is one of the most important factors to consider in controlling disease within zoos (Hosey et al., 2013), and there is sufficient evidence to suggest specific management practices may influence incidence of lumpy jaw in captive macropods (Burton, 1981). It is widely accepted that changes in husbandry may reduce the risk of exposure to pathogenic bacteria, and may be particularly effective in reducing incidence of lumpy jaw (Burton, 1981; Asperger et al., 2001; Jackson, 2003; Vogelnest, 2015). These changes include decreasing stocking densities, improving enclosure hygiene, and managing the delivery and content of the diet. Maintaining enclosure hygiene is challenging, especially due to the organic nature of many enclosure substrates, which may also provide an environment where pathogenic bacteria may thrive (Burton, 1981; Samuel, 1983; Ketz, 1997; Hosey et al., 2013). Various authors have stressed the need for strict hygiene in enclosures to reduce the risk of contamination of the environment (Burton, 1981; Smith, 1990; Vogelnest & Portas, 2008). Burton (1981) even suggested modifying the delivery of the diet (raising food off the ground to avoid faecal contamination), as well as modifications to the diet itself, to facilitate changes in faecal consistency which may result in more effective cleaning of macropod enclosures. There are conflicting recommendations regarding diet, with some studies reporting that soft diets do not maintain the integrity of the oral mucosa (Smith, 2009), whilst others suggest a more fibrous diet may create tougher mucosa (Eriksen, 1964; Wallach, 1971; Clarke, 2003; Smith, 2009); although the latter may result in mucosal penetration and therefore provide a route of entry for bacteria (Wallach, 1971; Clarke, 2003). Despite efforts by some zoological institutions to control diet and hygiene, their macropods continue to develop lumpy jaw.

Substrate

Enclosure substrate may provide a source of food, form part of the diet, but it may also harbour bacteria associated with lumpy jaw. Bennett et al. (2009) suggested that bacterial transmission between the ground and the mouth, via the faeces (during grazing), is likely to occur through unintentional or intentional coprophagic behaviour. However, lumpy jaw is acknowledged as being endemic in some enclosures and not in others (Le Souef & Seddon, 1929; Butler & Burton, 1980); suggesting that faecal contamination of ingested substrate is not the only factor involved in the development of the disease.

Climate

The captive environment is subject to the natural elements to a certain degree, and with macropods being housed in zoos worldwide, as shown in Table 1.2, climate, or the immunosuppressant effects of climatic stress (King & Bradshaw, 2010), may have a causative role in the development of lumpy jaw. A number of studies report a greater case incidence of lumpy jaw in captive macropods during colder periods (Burton, 1981; Oliphant et al., 1984; Kido et al., 2013), and during drought in wild macropods (Borland et al., 2012). These studies are also geographically and climatically diverse, providing evidence of climatic influence. However, the challenges associated with sub-optimal climate may also be a source of stress for macropods (King & Bradshaw, 2010; Hing et al., 2014); and stress is another factor known to be associated with lumpy jaw (Wallach, 1971; Burton, 1981; Vogelnest & Portas, 2008; Sotohira et al., 2017a; Sotohira et al., 2017b).

Stress

The captive environment creates an artificial habitat which may produce “potentially provocative environmental challenges” (Morgan & Tromborg, 2007, p. 262); these ‘stressors’ may affect biological and immunological function, which could contribute to the development and progression of diseases such as lumpy jaw (Blecha, 2000; Vogelnest & Portas, 2008; Hosey et al., 2013; Hing et al., 2014). A wide variety of ‘stressors’ that may be presented in the captive environment are reviewed by Morgan and Tromborg (2007), and include exposure to human contact, such as zoo visitors, artificial light, sound, temperature and odours. Environmental stress has previously been associated with the development of lumpy jaw, with overcrowding postulated as a potential source of stress (King & Bradshaw, 2010; Borland et al., 2012). The effects of population density and imposed abnormal social groupings, for example males and females being managed separately to control breeding (Hosey et al., 2013), can reduce an animal’s ability to perform important natural behaviours, and can also increase agonistic behaviours (Gansloßer, 1989; 1995; Höhn et al., 2000; Rushen, 2000; Gregory, 2004; Hosey et al., 2013); which can be causes of stress in captivity (Morgan & Tromborg, 2007; Blackett et al., 2017). In addition, animals maintained in zoos are likely to experience biological and psychological challenges such as exposure to novel pathogens and stressors, during transport to new institutions as part of breeding programs or for other reasons (Hosey et al., 2013). Stress, health and welfare are a linked continuum, influenced by several factors including genetics, age and past experiences (Hahn & Becker, 1984; Blecha, 2000; Meehan et al., 2016); all of which are subject to institutional differences in management practices. In this way, the risk factors for lumpy jaw may not just be environmental; they may also be host-specific.

1.4.5 Host factors

Genus and species

Lumpy jaw is thought to affect all macropods (Jackson, 2003), however the presence of the disease may be affected by genera- or species-specific differences in dental anatomy, diet and behaviour. Some species appear to be predisposed to the disease, including the red kangaroo, the eastern grey kangaroo, and the red-necked wallaby. Although these species are also some of the most commonly-occurring species within zoological collections, and frequently represented in the literature (Vogelnest & Portas, 2008), they are also species classified within the same genus, *Macropus*; therefore there may be genus-specific attributes which make these macropods more susceptible to lumpy jaw. There are few reports of lumpy jaw in the dorcopsises (genera *Dorcopsis* and *Dorcopsulus*), which may indicate species within these genera are less susceptible, or may instead reflect the lack of research on these genera (Arman & Prideaux, 2015). Genus- and/or species-specificity may also be the result of adaptation to captivity and loss of genetic variation, which can be seen in many small populations of species that are often found in zoos (Clauss et al., 2008; Kaiser et al., 2009; Ballou et al., 2010; Schulte-Hostedde & Mastromonaco, 2015). Some genera have behavioural traits that may influence the likelihood of developing lumpy jaw. Although macropods are prey species and prone to be nervous, they have differing flight responses (Jackson, 2003; Bond & Jones, 2014); therefore in captivity, some genera may be more susceptible than others to facial trauma as a result as fence-running. Species-/genus-specificity has been somewhat dismissed by Vogelnest and Portas (2008), however, given the differences in dental anatomy, as discussed earlier, and temperament between macropod species, it could be expected that some species may be more susceptible than others. For example, the flighty nature of some macropod species (Jackson, 2003) may increase the risk of fence-running resulting in facial trauma, and may increase the immunosuppressant effects of stress (Dohms & Metz, 1991; Blecha, 2000), thereby potentially increasing the risk of lumpy jaw in some species (Butler, 1981; Vogelnest & Portas, 2008).

Age

Macropod age has previously been hypothesised as having a role in lumpy jaw development, potentially as a result of the correlative relationship with molar progression (Clarke, 2003; Vogelnest & Portas, 2008; Kido et al., 2013). However, Borland et al. (2012) observed that bone lesions associated with lumpy jaw were rarely involved with erupting molars, and were instead involved with the more rostral teeth found within the oral cavity. Based on these findings it could be interpreted that age-related eruption of the molar teeth is not a common predisposing factor in lumpy jaw. Yet age is a factor in many diseases in other species, due to reduced immune function [e.g. dogs: (Alexander et al., 2018)]. The mean age of onset of lumpy jaw reported in one population of captive swamp wallabies is 3.1 (±2.1) years (Kido et al., 2013); this is relatively young for this species, given its expected longevity in captivity is often in excess of nine years (Jackson, 2003). However, the older the macropod, the longer the exposure time to potential risk factors for disease. Examples of age-related risks may include risks for older macropods exposed to an abrasive diet, leading to worn dentition (McArthur & Sanson, 1988; Christensen, 2014); conversely, longitudinal exposure to soft diets may have contributed to periodontal disease in older individuals, resulting in increased risk of penetration by coarse items in the diet (Finnie, 1976; Gamble, 2004; Vogelnest & Portas, 2008). Additionally, extended exposure to stressors (chronic stress) may increase the likelihood of lumpy jaw developing with time. Interpreting which aspects of captivity are contributory drivers of stress is essential to facilitate captive macropod health and welfare, and ultimately, to facilitate their conservation within zoos. Although age is likely to be a factor in the development of lumpy jaw, the effects of immuno-senescence (the deterioration of reproductive ability and fitness) may also be influenced by the sex of the macropod (Cockburn, 1997; Alexander et al., 2018).

Sex

The sex of an animal has been shown to influence the development and outcome of disease (Hing et al., 2017; Silk et al., 2017; Domínguez-Roldan et al., 2018). In some macropod species, sexual dimorphism in dentition has been noted; however, a sex bias in the development of lumpy jaw is yet to be detected (Vogelnest & Portas, 2008). Differences in behaviour, especially in feeding ecology, between male and female macropods may result in differences in dentition and tooth wear. Wild male macropods are reported to consume different types and quantities of food from females (Garnick et al., 2018), so differences may be expected to be observed in the incidence of lumpy jaw between sexes. In one study, foraging behaviour was found to be significantly greater in male parma wallabies, however these males were separated from females for management purposes, and therefore were not presenting their full behavioural repertoire (Rendle et al., 2018). Zoos frequently manage their populations to control breeding (Rees, 2011; Hosey et al., 2013), and in macropods, a greater number of females in an enclosure would be more representative of the sex ratio in mobs in the wild. However, the removal of males to create bachelor mobs can have negative effects for both sexes (Schulte-Hostedde & Mastromonaco, 2015); including by exposing males to an increased risk of competition and aggression from other males (Rendle et al., 2018). Information regarding host susceptibility, including sexual bias, needs clarification. However, the way in which macropods are managed varies between institutions, and potentially between countries. The search for the driving factors for lumpy jaw are a priority for those seeking to maintain a healthy population of macropods in captivity.

1.5 Significance of an epidemiological investigation of lumpy jaw

Management and husbandry practices can influence the health and welfare of captive animals (Hosey et al., 2013), and zoos worldwide use a combination of past experience and husbandry guidelines to inform keepers on the management techniques required to maintain a healthy environment for their animals. However, these guidelines are of variable quality, and recommendations for housing and husbandry are rarely compiled from scientific evidence (Melfi, 2009); which may increase the risk of development of disease (Hosey et al., 2013). The impacts that various management and husbandry practices have on animal health and welfare are well reported, however the specific factors associated with the development of lumpy jaw remain to be established.

This proposed study will provide an opportunity to measure levels of lumpy jaw in captive macropods, over time, and improve scientific knowledge about best practice in the care of macropods and treatment approaches used to manage lumpy jaw. In turn, this information may contribute to understanding the pathogenesis of lumpy jaw. The benefits of this knowledge may include new recommendations to decrease the morbidity and mortality rates associated with lumpy jaw, and to decrease the frequency of animal handling for diagnosis and treatment, with its associated stress. Fundamentally, this research will provide a deeper understanding of lumpy jaw; information that will be of benefit to the welfare of captive macropods worldwide.

1.6 Research Aims

The overall aim of the study was to determine the prevalence and incidence of lumpy jaw in captive macropods and identify host and environmental risk factors for the development of the disease. As a benchmark for disease levels in captive macropods, a secondary aim was to investigate the prevalence of lumpy jaw in wild macropod populations.

Therefore, this study aimed to:

- Determine the prevalence of lumpy jaw in macropods housed in zoos across two regions where macropods are popular exhibits: Australia and Europe.
- Investigate host, housing and husbandry risk factors associated with the development of lumpy jaw in captive macropods.
- Review treatments used to control and manage lumpy jaw, evaluating disease reoccurrence and survival of macropods.

- Investigate the prevalence of lumpy jaw in a wild population of western grey kangaroos and consider key variables of the free-living environment (e.g. population density) that may act as potential risk factors for macropods housed in captivity.

Identification of the specific risk factors associated with lumpy jaw is important for the clarification of disease pathogenesis (Burton, 1981; Bodley et al., 2005; Kido et al., 2013), and would assist with the development of preventive management strategies that could be implemented in zoological institutions. The results from this investigation will deliver a significant contribution to knowledge, providing macropod keepers and veterinarians with a practical framework for the prevention and management of lumpy jaw in a captive environment. The benefits of this will include new information to facilitate a decrease in morbidity and mortality rates associated with lumpy jaw, thereby improving the welfare of these iconic Australian animals housed in zoos worldwide.

1.7 Chapter Organisation

Following this introductory chapter, the general methods used in this research are discussed in Chapter Two, including: the development of a case definition, the criteria used for the selection of participating institutions, and the methodology used to extract data relating to lumpy jaw in captive macropods from zoo records. Thereafter, chapters comprise of individual studies of lumpy jaw in both captive and wild macropods, including a review of the treatment approaches and likely outcomes. Specifically, Chapter Three focuses on a survey of lumpy jaw in zoos across Australia and Europe, Chapters Four and Five concentrate on the retrospective epidemiological analysis of zoo and veterinary records from selected institutions, and Chapter Six examines the prevalence of lumpy jaw in wild macropods. Each chapter opens with a review of the literature, followed by specific methods used in each study. Subsequent results and discussion sections then follow. Chapter Seven collectively draws together results from each study and contextualises host and environmental triggers for lumpy jaw in captive macropods. This General Discussion chapter also makes recommendations for measures of controlling and managing lumpy jaw; essential information to achieve optimum health, welfare and the continued conservation of macropods in captivity.

Chapter 2

General Methods

2.1 Introduction

The objectives of this research were fourfold: firstly, to generate data that would determine the status of lumpy jaw in macropods housed in zoological institutions across the Australian and European regions; secondly, to identify housing and husbandry risk factors associated with the development of the disease; thirdly, to investigate treatments used to manage and prevent lumpy jaw; and finally, to generate data that would enable the disease status in a wild population of macropod species to be quantified. Initially, a cross-sectional epidemiological survey was used to ascertain the prevalence of lumpy jaw across two continents where macropods are popular exhibits in zoological collections. The survey also collected data regarding potential risk factors that may be associated with the development of lumpy jaw. A more detailed retrospective investigation of selected institutions was also undertaken, which aided in the expansion of causal hypotheses regarding the impact of housing and husbandry risk factors on the occurrence of lumpy jaw. The investigation of wild conspecifics, using skulls collected and stored from a previous management cull, provided baseline data from a wild population which could be used for comparative purposes when investigating lumpy jaw in captive macropods.

This chapter is comprised of three parts, outlining general methodological details relevant to the various epidemiological studies carried out to investigate lumpy jaw in captive and wild macropods.

- Part A provides an overview of the definitions developed for use throughout this research (Chapters 3, 4, 5 and 6);
- Part B describes the selection of the eight zoological institutions used in the retrospective epidemiological investigation (Chapters 4 and 5); and
- Part C details the methodology involved in the extraction of data relating to lumpy jaw cases from zoo records accessed for this study, and provides an overview of the analyses carried out with respect to the treatment of lumpy jaw over the retrospective period (Chapters 4 and 5).

2.2 Part A - Case definition

Lumpy jaw is a complex syndrome with multifactorial aetiology, and currently has no clear case definition. For the purposes of this study, we required a case definition that was likely to capture the majority of cases of lumpy jaw across the range of epidemiological investigations planned, but which could also be effectively used by others involved in this research.

2.2.1 Development of the case definition

The challenge of capturing true cases of lumpy jaw is that clinical lumpy jaw is considered to be the end stage of a continuum of oral disease (McLelland, 2019). Lumpy jaw progresses from gingivitis, in the early stages, through to periodontal disease, advancing further to involve the bones of the mandible and/or maxilla, whereby proliferation of the jaw is observed (McLelland, 2019). As the early stages of lumpy jaw are conditions in their own right, the development of a clear case definition was required, to differentiate lumpy jaw from other oral diseases. Aetiology of lumpy jaw is multifactorial, therefore the definition needed to be a compromise between being sensitive enough (broad enough) to pick up cases, but specific enough to avoid too many false positive cases. In addition, the case definition was also required to capture cases retrospectively, using clinical notes and diagnostics in zoo records, so the definition differed from what an ideal case definition would have been if a full work up had been performed for each clinical case, for example, using results from microbiology.

A case definition for lumpy jaw was developed using leading descriptors of the disease and tools commonly used in the diagnosis of lumpy jaw. The definition was circulated to a focus group of zoo professionals and veterinarians for feedback on sensitivity, specificity and clarity of the definition by zoo veterinarians and other personnel who may be involved in this research. Feedback was used to develop the final case definition, which was used to capture mid to later stages of the disease reported in clinical records.

2.2.2 Case definition

For the purposes of this study, we consider a case of lumpy jaw to be:

"*Proliferative bony change or soft tissue inflammation of the maxilla/mandible (lumps), and/or radiographic/visual evidence of osteomyelitis/osteolysis; accompanied by dental disease. There may or may not be demonstrable bacterial involvement through microbial culture.*"

In addition to other known terms used to describe lumpy jaw, such as oral necrobacillosis, as discussed in Chapter 1 (e.g. oral necrobacillosis), this definition is used throughout the thesis.

2.3 Part B - Selection of zoological institutions for retrospective investigation

The following section details the selection process for participation in the retrospective investigation of lumpy jaw (Chapter 4) and review of treatment approaches (Chapter 5). It also provides justification for the retrospective period selected.

2.3.1 Selection criteria

Species360 (until recently known as International Species Information System [ISIS]) is an international organisation that maintains a global database of online records pertaining to animals housed at zoological institutions worldwide. The online database, Zoological Information Management System (ZIMS), enables members of Species360 to centrally store husbandry and medical information for their collection animals. For the retrospective investigations in our study, we selected institutions, which, in addition to Species360 membership, maintained membership to professional zoological organisations. Membership of professional zoological organisations provides further assurance that member zoos and aquariums are seeking to achieve and maintain the highest standards of welfare for the species they keep, and to contribute to global biodiversity and conservation goals. Utilising zoos that hold professional membership(s) may also assist with the future dissemination of our research findings, which in turn will be of benefit to macropods housed in captivity. Professional zoological memberships held by our participating institutions included: World Association of Zoos and Aquariums (WAZA), Zoo and Aquarium Association (ZAA), European Association of Zoos and Aquaria (EAZA) and the British and Irish Association of Zoos and Aquariums (BIAZA).

Working in collaboration with one of the Species360 members, we obtained access to ZIMS, which enabled the generation of the reports required for this research. Species Holding Reports (SHR) were extracted for genera under the Family 'Macropodidae' (*Dendrolagus*, *Dorcopsis*, *Lagorchestes*, *Onychogalea*, *Macropus*, *Petrogale*, *Thylogale*, *Setonix* and *Wallabia*) for two regions, Australia and Europe. The SHR facilitated the selection of zoos based primarily on their current ownership of the western grey kangaroo (*Macropus fuliginosus*), the focal species for another aspect of the research, which involved retrospective examination of skulls to determine the presence of lumpy jaw in two wild populations of western grey kangaroos which had been culled for population control measures (see Chapter 6). Institutions were also selected based on knowledge of previous history of lumpy jaw from anecdotal/personal communication, or literature-based evidence, and on existing established links with Murdoch University staff involved in this study. We also included institutions that had previously expressed an interest in participation in the study. To increase sample sizes, zoos were preferentially targeted if, during the time of this research, they held a range of macropod species and in relatively high numbers. It was observed that some zoos housed endangered macropod species, therefore their inclusion in the study was prioritised, as optimising the captive care of these species could be critical to the survival of the species.

To limit zoo 'type' becoming a confounding factor, we selected for modern, urban zoos, geographically positioned near to town and cities. We did not include institutions that were described as open range or safari-type zoos. To assess zoo type, we reviewed the websites of institutions reported on ZIMS as being current holders of macropods (if the institution was not already known to a member of the research team). To minimise the effect of climatic differences becoming a major confounding factor, geographical location was also considered. Attempts were made to work with institutions that were closely situated, however this was challenging in the Australian region. Nevertheless, this provided an opportunity to investigate potential links between health and climate.

2.3.2 Selected institutions

In total eight zoos were selected; four from Australia, and four from Europe; three in the United Kingdom and one in Switzerland.

The eight institutions that participated in the retrospective cohort study were anonymised for privacy. From here, institutions in the Australian region are identified with the prefix 'A' and individually numbered 1 to 4. European institutions are identified with the prefix 'E', and are also individually numbered 1 to 4.

Zoos were invited, via email and a formal letter sent as an attachment (Appendix A), to participate in a retrospective investigation of lumpy jaw in captive macropods. A request was made to access current and historic veterinary and husbandry records during a two-week visit to each institution. Until institutional access to the ZIMS database was granted, and zoo records could be extracted, the exact population of macropods housed at each institution during the retrospective period was unknown.

2.4 Part C - Data extraction

2.4.1 Retrospective period

The retrospective period was selected to incorporate any changes that may have occurred in housing or husbandry in the zoos over recent decades, and to provide the greatest opportunity to collect larger quantities of data. Similar to approaches adopted by other researchers (Kido et al., 2013), a retrospective period of 20 years was selected, to capture an average lifespan across several species of macropods (reviewed in Jackson, 2003). The retrospective study period used in the calculations of prevalence and risk factor analyses, 1st January 1996 to 31st December 2015, pre-dated the standard use of computerised record keeping; therefore, direct visitation to each of the zoos was required, to collect data from paper-based records. In addition, online access to clinical records was granted through each institution, which was subject to a limited period; namely, only while the researcher was present at the zoo. Chapter 4 has an explanatory note regarding a difference in the retrospective period for incidence rate calculations.

Prior to visits to the institutions, we requested institution-specific Taxon Reports (accessed through ZIMS), for all nine macropod genera housed at that institution from 1st January 1994 through to the date of the request; this date was later trimmed for prevalence and incidence rate calculations (see Methods in Chapter 4 pp. 115 - 117). The Taxon Reports provided animal species, numbers housed, sex, and any inter-zoo transfers for the retrospective period in question, in addition to Global Accession Numbers (GAN) and Local Identification number, used to assist with the location of each individual's clinical records. Data from Taxon Reports were entered into Microsoft® Excel 2016 (Microsoft® Corporation, Washington, USA).

2.4.2 Extraction of electronic animal and medical data

Institutions used a variety of methods for the storage of biological, clinical, housing and husbandry data; therefore a wide variety of records were utilised to collect information of clinical lumpy jaw disease, and history of housing and husbandry. Not all records were complete, therefore all records available were collected, in order to generate the best opportunity to find missing information by examining other records. Prior to the commencement of the data collection, extensive ZIMS training was undertaken at Zoo A3, to ensure the full collection of records could be located and accessed.

Prior to January 24th January 2016, ZIMS reports were run using ZIMS Version 2.2 (released 29th June 2015). Following this date, records were extracted from ZIMS Version 2.3 (released 25th January 2016). With institutional access provided on arrival at each zoo, the following records were retrieved from ZIMS:

Taxon Reports

After gaining institutional access to ZIMS, specific selections were made to capture only the 'Local' animals that were part of the 'Main Institution Animal Collection', as well as those that were 'Owned and On Site' between our selected dates (1st January 1995 to current date). Each taxonomic genus was selected in turn, and reports were generated, downloaded and saved as pdf files. Historically, numerous hybrids have occurred in zoos (Jackson, 2003), one of which was detected in the records search. As a *Macropus* hybrid, this individual was included in the study as it fitted the selection criteria (to be discussed), but it was not included in the species-specific analyses.

Specimen Reports

Specimen Reports (SR) contained data relating to life history, current location (if still alive and present in the zoo), sex, birth, rearing method, inter- and intra-zoo transfers, some death information and enclosure history. Reports were generated using the GAN for each individual macropod, and the function to 'Select all' was utilised. The date range for the report was selected from 1st January 1800 to current date, as this enabled an animal's full life history to be found. Each report was run, downloaded and saved to pdf for later review.

Notes and Observations

The 'Notes and Observations' section in ZIMS was generally used by keepers to diarise information regarding changes in animal behaviour, diet or enclosure environment (general husbandry). However, this section was also heavily used for the reporting of health issues and subsequent medical treatments at some institutions. These records were a good source of secondary information to clarify missing dates or obtain information that may have been missing from other records, and they were also the most heavily used of all the records in most institutions.

The Notes and Observations were located by first doing a simple 'Animal' search using the individual's GAN. This leads to a page where there are several sections of information. The 'Notes and Observations' was one of these sections, and was selected to open the page. All the data relating to a particular animal could then be downloaded and saved to pdf.

Enclosure Reports and Animal Reports

Enclosure reports provided information on animal location within the zoo. The enclosure reports were run in the same way as the other reports; by requesting the enclosure's given identification code for the selected years. Not all zoos utilised this ZIMS module, therefore it proved to be an ineffective method for extracting information. However it did afford the opportunity to check enclosure details if they were not recorded elsewhere.

Medical records (ZIMS and Filemaker)

Medical records provided information on the diagnosis of lumpy jaw, date of diagnosis, period of treatment, treatment used, any surgical intervention, and an outcome, with associated date. ZIMS Medical Version 2.2 was used for the extraction of clinical records. On selection of the Medical Mode, a further selection was made for 'Full Medical History'. The GAN for each individual animal was entered, and a selection was made for 'All animals' (this enabled non-current animals to be located), and the report was generated. For each animal for which a medical history was available, the report was downloaded and saved as a pdf for later review.

Zoo E1 recorded their clinical data using FileMaker® Pro software (Filemaker, Inc., Santa Clara. Version Pro/Server 9*). The records were found on the system by searching 'Graues Riesenkänguru' (western grey kangaroo). This search enabled the researchers to locate the files of all macropods that had been housed at Zoo E1 since Filemaker was introduced. Zoo E1 had been using Filemaker since 2000, therefore all records before this date were paper-based. Google® Translate (Google® Inc., Mountain View, California, USA) was used to translate all information from German into English. Acronyms that could not be translated through online means were confirmed by conversing with the veterinary team at Zoo E1.

2.4.3 Extraction of paper-based records

All the zoos included in this study held some paper-based records. These included several years of clinical records (some of which had been entered into ZIMS), necropsy reports, keeper records, diet sheets, zoo and enclosure maps. Paper records that were dated within our study period, were scanned using a handheld Epson® WorkForce DS-40 scanner (Epson® Australia Pty Ltd., North Ryde, Australia) and saved as pdf copies for later reviewing.

Additional methods specific to each chapter are discussed applicably elsewhere in this thesis.

2.5 Ethics approval

Ethics approval for this study was given by the Murdoch University Animal Ethics Committee (Permit Number R2754/15) and Human Ethics Committee (Permit Number 2015/182) Participating institutions provided their own approval where necessary (Zoo A2: ZV16005; Zoo A3: 2015-6; Zoo A4: R16D217).

Chapter 3

Epidemiological survey of captive macropods to determine prevalence and risk factors for lumpy jaw

3.1 Introduction

Lumpy jaw is a disease that affects captive macropods worldwide, and is considered a significant health threat to captive macropods (Vogelnest & Portas, 2008). Despite the apparently high incidence of this disease in captivity, limited epidemiological data have been published examining the true prevalence and risk factors associated with the development of clinical disease. Lumpy jaw is frequently associated with captivity, and in particular hypothesised risk factors include an artificial diet (Finnie, 1976; Brookins et al., 2008), feeding strategies (Burton, 1981), high stocking densities, and associated enclosure contamination with microbial agents found in faeces (Calaby & Poole, 1971; Burton, 1981; Ketz, 1997) and stress (Butler & Burton, 1980; Ketz, 1997; Staker, 2014). Our understanding of the influence of housing and husbandry of zoo animal health is often based upon tradition rather than empirical evidence, and a multi-institutional survey of housing and husbandry practices may help to identify specific practices that influence the incidence and prevalence of this disease.

Macropods are popular exhibits in zoos, with records indicating that at the time of writing (22nd October 2018), 6886 individuals were housed across 469 registered zoological institutions worldwide (Species360, 2018). The two most popular regions are Australia and Europe, where nearly three quarters of the world's captive macropods are housed (Australia 25.7%, n = 1768; Europe 48.8%, n = 3360) (Species360, 2018). It should be noted that these figures underestimate the true number of macropods held in captivity, as they do not include macropods housed in privately owned collections, or those institutions that are not subscribed members of Species360. Reports in the literature of prevalence of lumpy jaw in captivity are limited to a small number of studies, however, the reported prevalence ranges from 0 – 100% (Butler & Burton, 1980; Vogelnest & Portas, 2008; Kido et al., 2013), indicating that a large number of macropods housed in institutions across Australia and Europe could potentially be affected by this debilitating disease (Table 1.5).

Lumpy jaw represents a continuum of changes to the periodontal structures and the jawbones, most often characterised in the advanced stages by proliferative swellings of the maxilla and mandible, in association with the presence of a range of bacterial species (Burton, 1981; Samuel, 1983; Oliphant et al., 1984; Antiabong et al., 2013a; Antiabong et al., 2013b). It has been suggested that disease occurs when pathogenic bacteria penetrate the soft tissue of the oral cavity, causing suppurative lesions of the soft tissue and bone, which can lead to osteomyelitis in chronic infections (Wallach, 1971; Brookins et al., 2008; Vogelnest & Portas, 2008). A long-established belief is that oral trauma, caused by products within the diet, may be responsible for the introduction of bacteria into the periodontal structures, thereby initiating the process of infection and development of lumpy jaw (Beveridge, 1934; Finnie, 1976; Arundel et al., 1977; Munday, 1978; Clarke, 2003). Consequently, recommendations have been made to remove sharp grasses from the macropod diet, to reduce this risk (Wallach, 1971; Jackson, 2003). However, Burton (1981) observed that macropods fed on a coarse diet did not always experience trauma to the oral mucosa. He also reported that feeding a soft, less fibrous diet with a greater carbohydrate content, resulted in changes to the gingiva caused by the proliferative development of dental plaque associated with a high carbohydrate diet. It is these changes that may lead to reduced resistance to oral trauma; a hypothesis raised earlier by Finnie (1976). The feeding of soft diets reduces masticatory effort (Lentle et al., 2003; Vogelnest & Portas, 2008) and is postulated to prolong the shedding of molar teeth; thereby affecting normal molar progression, another potential risk factor for lumpy jaw (Finnie, 1976; Clarke, 2003).

Methods used to deliver macropod diets vary by institution and enclosure type, and may increase the likelihood of faecal contamination of the diet, particularly where scatter feeding is carried out, or where macropods ingest feed that has fallen to the ground (Burton, 1981). As suggested by Bennett et al. (2009), faeco-oral transmission of bacteria is possible during grazing, including through unintentional or intentional coprophagic behaviour. Various techniques have been recommended to reduce faecal contamination of enclosures, and therefore feed; including reducing stocking densities, improving enclosure hygiene, and raising feeding platforms off the ground (Burton, 1981; Wallach & Boever, 1983; Vogelnest, 2003). However, the specific influence of such changes in management on lumpy jaw incidence and prevalence has not been investigated.

The congregation and high-density housing of macropods in captivity are considered to create an environment conducive to the development of lumpy jaw (Butler, 1981; Vogelnest & Portas, 2008), due to the influence of increased stocking densities on faecal contamination as well as stress (Burton, 1981; Ketz, 1997). Several researchers have implicated periods of acute stress as a potential factor in the occurrence of lumpy jaw in macropods (Finnie, 1976; Vogelnest & Portas, 2008; Borland et al., 2012). Multiple stressors usually exist in captive environments, which can result in negative impacts on the individual's behaviour, autonomic nervous system, neuroendocrine system and immune responses (Moberg, 1985; Moberg, 2000). Physiological responses to stressors include the activation of the hypothalamic pituitary adrenal (HPA) axis, which aims to maintain normal biological functioning (homoeostasis). Overstimulation of the HPA axis can result in pathological changes in the body that have been implicated in immunosuppression (Terlouw et al., 1997); although, as reviewed in Martin (2009), the role of stress in immune function is complex, variable and poorly understood. In the captive environment, sources of stress may be related not only to stocking densities, enclosure types and content (including animals housed within), but also to other housing and husbandry variables including visitor presence/ interaction (Hosey, 2000; Hosey & Melfi, 2015), inter- and intra-zoo transfers; and within-enclosure lights, sounds, odours and temperatures (climate) (Calaby & Poole, 1971; Herter et al., 1985; Ord et al., 1999; Miller, 2001; Gregory, 2004; Hillmann et al., 2004; Marai & Rashwan, 2004; Rees, 2004; Morgan & Tromborg, 2007). The challenge is to identify the potential sources of stress within a macropod's environment, as well as to determine the biological mechanism(s) of stress in relation to lumpy jaw. As with other potential risk factors for this disease, our understanding of the relationships between these stressors and the development of lumpy jaw, and consequently the associated management recommendations required to reduce both, are at present largely theoretical rather than evidence-based.

Climate has been suggested as another risk factor for the development of lumpy jaw, based on reports of differences in prevalence of the disease between institutions that have different climatic conditions. In one example, Kido et al. (2013) carried out a retrospective cohort study (1987 - 2011) of swamp wallabies at a Japanese institution, and reported a higher prevalence of lumpy jaw (P = 40.7%, 95% CI: 27.57 – 54.97) compared to the prevalence reported by Vogelnest and Portas (2008) for swamp wallabies at an Australian institution (P = 9.5%, 95% CI: 4.20 – 17.91, $p < 0.0001$). However, this comparison may be biased by the inclusion of both captive and wild swamp wallabies in the latter study. Climate-related and seasonal trends in lumpy jaw detection have also been reported by Burton (1981) and Oliphant et al. (1984); with a higher reported occurrence of lumpy jaw during the cooler, wetter winter months at zoos in both Australia and the United Kingdom. Ketz (1997) also noted that particular substrates may harbour pathogenic bacteria in wetter geographic regions. However, any geographic and climate-related differences will likely be confounded by institutional management variables, including housing type, substrate, feeding practices, species assemblages, and other institutional differences, which may influence susceptibility.

Despite macropods varying in size, dental anatomy, grazing strategies, diet, behaviour and responses to the captive environment (Jackson, 2003; Vogelnest & Portas, 2008; Staker, 2014), lumpy jaw has been in detected in most captive macropod species (refer to Table 1.5 in Chapter 1, p. 27). However, it is most frequently reported in three species: red-necked wallabies, and red and grey kangaroos (Brookins et al., 2008; Vogelnest & Portas, 2008). This may be related to the popularity of these species within collections (Vogelnest & Portas, 2008). Species-specific susceptibility to lumpy jaw has not been systematically investigated.

Scientific reporting of lumpy jaw in captive environments frequently takes the form of case studies, usually related to specific treatment modalities (e.g. Brookins et al., 2008; Bakal-Weiss et al., 2010). Few epidemiological studies of lumpy jaw have been undertaken, and where prevalence data are reported, they are primarily from a single institution such as studies undertaken by Vogelnest and Portas (2008) and Kido et al. (2013). Prevalence figures may also be based on identification of lumpy jaw at necropsy such as prevalence data reported by Vogelnest and Portas (2008). This risks misrepresenting the true prevalence, and also omits individuals that survive the disease. Kido et al. (2013) conducted a retrospective cohort study of lumpy law in swamp wallabies at a Japanese zoo and reported a high (40.7%) prevalence of the disease. Other differences between the Vogelnest and Portas (2008) and Kido et al. (2013) studies included species (many versus single) and use of point prevalence (proportion of disease in a population at a particular point in time such as at necropsy), versus period prevalence (proportion of new cases that develop in a population recorded over a period of time, for example, 5 years). The differences in approach between studies limit the comparisons that can be made. Furthermore, while retrospective studies such as those conducted by Vogelnest and Portas (2008) and Kido et al. (2013) may be beneficial in capturing potential risk factors for disease at a single institution, they cannot provide an indication of the prevalence regionally or globally. To investigate the prevalence of lumpy jaw in captive macropods across regions, and to identify risk factors, a multi-zoo study would be required.

One published multi-zoo study (Ketz, 1997) reviewed the relationships between variables in the captive environment, and the occurrence of lumpy jaw. This study, a retrospective study of necropsy records, was unable to determine a relationship between aspects of the captive environment and incidence of lumpy jaw; but findings were limited because retrospective housing and husbandry data were unavailable, so the historic necropsy data could only be correlated with current housing and husbandry data (Ketz, 1997). The author noted that changes in husbandry management conditions had occurred over the preceding decade; which may have been related to the observed decline in prevalence of lumpy jaw in the institutions studied (Ketz, 1997). Although multi-zoo studies such as the Ketz (1997) study, and this present research, may face challenges associated with the introduction of additional confounding variables (Hosey et al., 2013), multi-zoo research has important advantages. For example, it provides an opportunity to investigate the effects of variables across institutions, and critically, it allows the determination of prevalence of diseases, such as lumpy jaw, at regional and global levels.

Extending zoo-based research to include multiple institutions enables investigation into a possible relationship between the differences between institutions, and differences in the health and welfare of their animals. Multi-zoo research has typically relied on the use of surveys (Hosey et al., 2013), which are a common data collection method in epidemiological research, and, are used frequently in zoos for the collection of both epidemiological and other data (Plowman et al., 2013; Thrusfield & Christley, 2018). Zoo-based research is often criticised due to the resultant small sample sizes (Hosey et al., 2013). Surveys have the advantage of generating large quantities of data without the need for direct intervention of the animals' environment; providing an opportunity to increase sample sizes without impacts to animal welfare (Hosey et al., 2013; Plowman et al., 2013; Thrusfield & Christley, 2018). In addition, surveys provide an opportunity for a greater geographical area to be covered with minimal time and cost involved (Thrusfield & Christley, 2018). When executed well, surveys can be cost effective in gathering data from multiple zoological institutions (Rees, 2011).

A peer-reviewed literature search identified several multi-institutional questionnaire surveys that have been used across taxa in zoo-based research; however, very few of these surveys included a focus on macropods. In one example, a multi-zoo questionnaire survey, carried out by Boulton et al. (2013), successfully extracted data relating to prevalence, causative agents and risk factors for dermatophytosis in macropods. Macropod-specific questionnaire surveys are more commonly developed for studies of wild macropods. In one early study, Tomlinson and Gooding (1954) conducted a postal survey to obtain temporal and spatial data regarding an outbreak of lumpy jaw in wild kangaroos in Western Australia. Although their study did not determine the prevalence of lumpy jaw, it did provide valuable information regarding the clinical signs of the disease. One potential issue for the Tomlinson and Gooding (1954) study may have been the presence of 'respondent bias', through the specific targeting of pastoralists affected by kangaroos competing with livestock for grazing. This questionnaire formed part of a larger investigation into the potential use of biological methods to control kangaroo populations in the region, and, included the deliberate infection of kangaroos with bacteria associated with lumpy jaw. It is likely that respondents had a personal interest in completing the questionnaire, as the results of the survey would lead to a kangaroo control program in the region, which would ultimately have a positive effect on their livelihoods as pastoralists.

Uncontrolled bias is a commonly observed flaw of survey-based zoo research (Van Gelder et al., 2010; Thrusfield & Christley, 2018). One way that bias can be introduced is through survey type; for example, online surveys have become common in epidemiological research (Van Gelder et al., 2010; Thrusfield & Christley, 2018), but in zoos, some target groups, such as keepers, may not have equal access to computers to complete such surveys (Plowman et al., 2013). The method of survey delivery can also affect response rates (Rindfuss et al., 2015), and needs to be considered carefully during study design.

Surveys have many limitations and challenges that must be managed at the design and implementation stages, associated with (for example) detail of data, reliability, and issues around bias (Christley, 2016; Thrusfield & Christley, 2018). However, when well designed and implemented, surveys can make an important contribution to existing knowledge. In the case of lumpy jaw, use of surveys can identify knowledge gaps, drive hypotheses for future research, and potentially provide guidance for the future management of this disease.

3.2 Aims

Lumpy jaw is widely considered a problem of captivity, potentially related to a range of husbandry and management variables (Wallach, 1971; Jackson, 2003; Vogelnest & Portas, 2008; Vogelnest, 2015). Many differences in the husbandry and management of macropods will exist between different captive institutions. Given this, our study aimed to investigate relationships between management practices and the prevalence of lumpy jaw, by capturing macropod management and health data from a large number of institutions. We aimed to describe the prevalence of lumpy jaw in Australian and European zoological collections; capture information on the captive management of macropods, including diagnostic and treatment options used in the management of this disease; and investigate links between prevalence and management variables (potential risk factors). Understanding the epidemiology of lumpy jaw in captive macropods, including risk factors associated with its occurrence, is fundamental to the development of recommendations for disease prevention and management.

3.3 Methods

3.3.1 Ethics approval

Ethics approval for this study was provided by Murdoch University's Human Ethics Committee (Project Number 2015/182). A number of the zoological institutions in our study also required individual research approval for the collection of survey data, including Bristol Zoological Society, Colchester Zoo, Marwell Zoo, and Welsh Mountain Zoo – National Zoo of Wales; institutional approval for the study was advised by email.

3.3.2 Survey development

A survey was developed in English during 2015, to be distributed to zoological institutions across Europe and Australia between March and August 2016. Care was taken to ensure that all questions in the survey were short, simple, and clearly written, to minimise unintentional bias or 'leading questions', and to assist those where English was not a first language (see Appendix B for survey). Questions were presented in a variety of formats, including tables, drop-down selections, and with areas where respondents could add their own comments. During survey development, a draft survey (in Microsoft® Word 2016) was given to a selected group of zoo veterinarians, zoo and social scientists, epidemiologists, zoological association research committees, members of the Marsupial and Monotreme Taxon Advisory Group, and lay persons, to review the survey questions, structure and style, and provide feedback on the survey's appropriateness and ease of its use. The feedback obtained led to refinements to the survey, and the final version of the survey was subsequently distributed online in March 2016.

The online version of the survey was developed in conjunction with Murdoch University Application Support Analysts and was hosted on the Murdoch University server. The survey was designed to be distributed via a link connected to a specific institutional email address, and responses were linked to this individual email address. This system enabled the survey results to be automatically formatted into Microsoft® Excel for later analysis.

3.3.3 Sourcing participants

To source institutions to participate for our study, we used the ZIMS database to identify member institutions across the Australian (Oceania) and European regions that were currently housing macropods. Where email addresses were not readily available from the ZIMS database, internet searches were used to find this information.

Zoological institutions receive a large number of requests for completion of surveys (Plowman et al., 2013); therefore, formal support for this study from zoological associations and other relevant groups was invaluable. Associations and groups which were contacted and offered support for the survey included: Zoo and Aquarium Association (ZAA); European Association of Zoos and Aquaria (EAZA); the British and Irish Association for Zoos and Aquariums (BIAZA); Stud Book Keepers; Verband der Zoologischen Gärten e.V.; Australian Mammals Veterinary Advisory Group (VAG); Veterinary Specialist Advisory Group (VetSAG); and the European Marsupial and Monotreme Taxon Advisory Group (TAG). Where no email was available publicly, an online request form was submitted to the institution, introducing the project and requesting the institution's assistance in the completion of a survey. A request was made for a direct email address of the most appropriate person to whom the questionnaire should be sent. BIAZA awarded the project an official letter of support from their Research Committee, which provided recognition that the survey was supported by this official organisation (Appendix C).

3.3.4 Delivery of the survey

An email was sent to institutions and potential participants two weeks prior to the distribution of the survey, requesting the direct email of the most appropriate person to complete the survey. This email was supported by information regarding the importance of the study, the Participant Information Statement (Appendix D), and the BIAZA letter of support. The survey was then distributed via email on 31st March 2016, with an initial deadline of 31st May 2016 (Round 1).

Due to technical problems experienced with the online version, a data-enabled pdf version which could be completed with typed or hand-written responses (from here on referred to as paper-based), was re-distributed on 11th April 2016. A reminder email was sent in mid-May 2016. To encourage a greater uptake of participants, the closure date was extended until 31st July 2016 (Round 2) with a final reminder email, together with a paper-based copy of the survey attached, distributed on 25th July 2016. The survey was closed on 31st July, with a blanket email of thanks sent on 1st August 2016.

3.3.5 Survey content

The survey was comprised of two sections, totalling 25 questions. The survey aimed to investigate the prevalence of lumpy jaw in macropod species housed in institutions across Australia and Europe, and to assess common practices of housing and husbandry (refer to Appendix B for survey). The first section of the survey was comprised of 12 questions, designed to establish institutional location and current macropod housing and husbandry practices. Section two was comprised of 13 questions, intended to ascertain prevalence, diagnosis and treatment of lumpy jaw within the preceding five-year period. Biological characteristics, such as species and sex, were requested for those diagnosed with lumpy jaw. To assist with later data analyses, macropod age was categorised as 'adult' or 'juvenile'. Variability in sexual maturity between macropod species and also between the sexes required institutional discretion for the provision of information relating to macropod age. As the survey was sent out in early 2016, we assumed the data recorded were for the years 2011 to 2015. Participating institutions with no recorded incidence of lumpy jaw over the preceding five-year period were able to skip most of this section, with the exception of the questions relating to general institutional veterinary support. Within each section, there was the ability to add further comments.

3.3.6 Data processing

Data from the online survey were automatically generated into a Microsoft® Excel 2016 spreadsheet, with modification and cleaning required for analyses (see below). Data from paper-based surveys were manually entered into Microsoft® Excel 2016 and descriptive statistics were calculated. Confidence intervals were calculated in Microsoft® Excel 2016 using the exact binomial method (Ross, 2003). Statistical analysis and the calculation of odds ratios (OR) were performed using Epitools (Sergeant, 2018). Measures of difference for categorical data, which included geographical region, sex, age and frequency of veterinary support and biosecurity, were assessed using the Chi-square test where samples sizes were > 5, as calculated in Epitools (Sergeant, 2018). Where sample size in a single category was < 5, the two-tailed Fisher's exact test was used.

In cases where institutions had completed the survey multiple times, for example, when surveys were completed per species rather than per institution, or other repetitions were discovered, data were cleaned so that there was a single set of data for each institution. Categorical data were grouped together to simplify statistical analysis (Table 3.1). Data that were illegible (illegible hand writing on paper-based surveys) or invalid (where the numerator was greater than the denominator), were removed prior to statistical analysis.

Table 3.1: Revised categorisation of variables 'feeding methods', 'frequency of veterinary presence' and 'frequency of health assessments', to enable simplified statistical analysis.

Category	**Original categorisation**	**Revised categorisation**
Feeding methods	Scatter/ground feed	Scatter
	Trough/bowl on the ground	Ground
	Trough/bowl raised off the ground	Raised
	Hand feed	Hand
	Individual bowl	Other
	Other	Other
	> 1 method reported	Mixed (> 1 method reported)
Frequency of veterinary presence	As required	As required
	Daily	Daily
	Two or more visits weekly	≥ 2 visits weekly
	One or more visits monthly	≥ 1 visits monthly
	Less than one visit per month	< 1 visit per month
	Mixed	> 1 method reported

Category	Original categorisation	Revised categorisation
Frequency of health assessments	As required	As required/opportunistically
	Opportunistically	As required/opportunistically
	Daily	Daily
	Every 3 - 6 months	3 - 6 months
	Every 1 - 2 years	12 - 24 months
	Every 3 - 5 years	36 - 60 months
	Less than every 5 years	60 months+
	Mixed	Mixed (> 1 method reported)

N.B. *Scatter* – scatter feeding and the feeding from bowl/trough placed on the ground enabling faecal contamination; *Raised* – trough/bowl/net or other method of raising item off the ground; *Hand* – fed directly from the hand of a keeper or visitor (unclear as to which); *Mixed* – responding institution reported more than one method used. Institutions completed the sections relevant to themselves; therefore n varied for each component. *Other* – diet fed using individual bowl or by other means not recorded.

3.4 Results

3.4.1 Respondents

Respondents and response rates

A total of 527 institutions across Australia and Europe were emailed with a link to the online survey. We received automated reports that 50 were undelivered/ undeliverable, therefore 477 institutions were believed to have received the email link for the survey. We received responses from 20.1% (96/477) of institutions contacted, with 16.8% (80/477) providing sufficient information to be subject to data analysis. Not all institutions answered every question in the survey, so sample sizes for specific questions or sections of the survey are indicated in the results below.

Results showed that 42.5% (34/80) of responding institutions completed the online survey, whilst 57.5% (46/80) completed the paper-based version.

Respondent profession

The greatest number of respondents reported their profession as 'Veterinarian', 37.5% (30/80); whilst veterinary nurses were the lowest responding profession 2.5% (2/80). Other zoo professionals who completed the survey, under the category of 'Other', included: zoo manager, director, curator, non-macropod keeper, veterinary student, intern (no further description given), biologist, Chief Executive Officer (CEO), and research staff. Nine of the 80 surveys (11.3%) were completed by more than one professional (Mixed Professions) (Table 3.2).

Table 3.2: Profession of staff member(s) from Australian and European zoological institutions who responded to an online survey relating to institutional cases of lumpy jaw in their macropods (2011 - 2015).

Profession	Percentage of respondents %	Number of respondents
Veterinarian	37.5	30
Other	35.0	28
Mixed professions	11.3	9
Registrar	7.5	6
Macropod Keeper	3.8	3
Veterinary Nurse	2.5	2
Undetermined	2.5	2

Geographic distribution

A total of 19 countries were represented by responding institutions, with 10% (8/80) of institutions from Australia and 90% (72/80) from Europe. The greatest number of responding institutions were based in the United Kingdom, 22.5% (18/80), and eight countries had only one institution respond to the survey (Table 3.3).

Table 3.3: Geographic location of responding institutions completing a survey of institutional cases of lumpy jaw in their macropods diagnosed 2011 – 2015.

Country	**Percentage of respondents %**	**Number of respondents**
Australia	10.0	8
Austria	2.5	2
Belarus	1.3	1
Belgium	3.8	3
Cyprus	1.3	1
Finland	1.3	1
France	15.0	12
Germany	20.0	16
Hungary	1.3	1
Ireland	1.3	1
Italy	2.5	2
Lithuania	1.3	1
Netherlands	3.8	3
Poland	2.5	2
Russia	3.8	3
Slovakia	1.3	1
Spain	1.3	1
Switzerland	3.8	3
United Kingdom	22.5	18

3.4.2 Lumpy jaw prevalence

Region and country

All 80 (100%) responding institutions identified the presence or absence of lumpy jaw in the last years (2011 - 2015); providing regional and country-level data.

A total of 71.3% (57/80) responding institutions, from across 16 countries, reported one or more cases of lumpy jaw in the last five years. Twenty-one of these institutions (36.8%) identified cases of lumpy jaw in more than one species at their institution. Prevalence estimates were calculable for 94.7% (54/57) responding institutions, which provided both the number of macropods affected with lumpy jaw during the five-year period and total macropod population data.

The period prevalence of lumpy jaw in macropods housed in zoos across the Australian and European regions collectively (2011 – 2015) was 17.4% (95% CI: 15.7 - 19.5, n = 1598). The period prevalence of lumpy jaw in the Australian region was 12.6% (95% CI: 9.9 - 15.7, n = 541). This was significantly lower than that of the European region (P = 19.9%, 95% CI: 17.5 - 22.4, p = 0.0003, n = 1057). Odds ratios found European macropods were 1.7 times more likely to have lumpy jaw than their Australian housed macropods (OR 1.72; 95% CI: 1.28 – 2.32, p = 0.0003, n = 1598).

Although lumpy jaw was reported from 16 countries, population data to enable prevalence calculations were only available from 14 countries. The prevalence of lumpy jaw in captive macropods ranged from 5.9% (95% CI: 0.7 – 19.7, n = 34) in Switzerland, to 33.3% (95% CI: 4.3 - 77.7, n = 6) in Italy (Figure 3.1). Compared to Australia, the prevalence of lumpy jaw was significantly greater in the following countries: Belgium (P = 30.4%, 95% CI: 13.2 – 52.9, p = 0.01, n = 23), France (P = 19.2%, 95% CI: 15.2 – 23.8, p = 0.007, n = 338), Germany (P = 28.0%, 95% CI: 21.2 – 35.7, $p <$ 0.0001, n = 157), Hungary (P = 31.3%, 95% CI: 16.1 – 50.0, p = 0.003, n = 32) and the Netherlands (P = 29.4%, 95% CI: 17.5 – 43.8, p = 0.0009, n = 51). Macropods housed in institutions in Hungary were at greatest risk of being diagnosed with lumpy jaw compared to those in Australia (OR 3.16, 95% CI: 1.44 – 6.96, p = 0.003) (Table 3.4). Within the European region, one significant difference between countries was observed: macropods housed in institutions in Germany were 6.2 times more likely to have lumpy jaw than those in Switzerland (OR 6.23, 95% CI: 1.43 - 27.11, p = 0.004, n = 191).

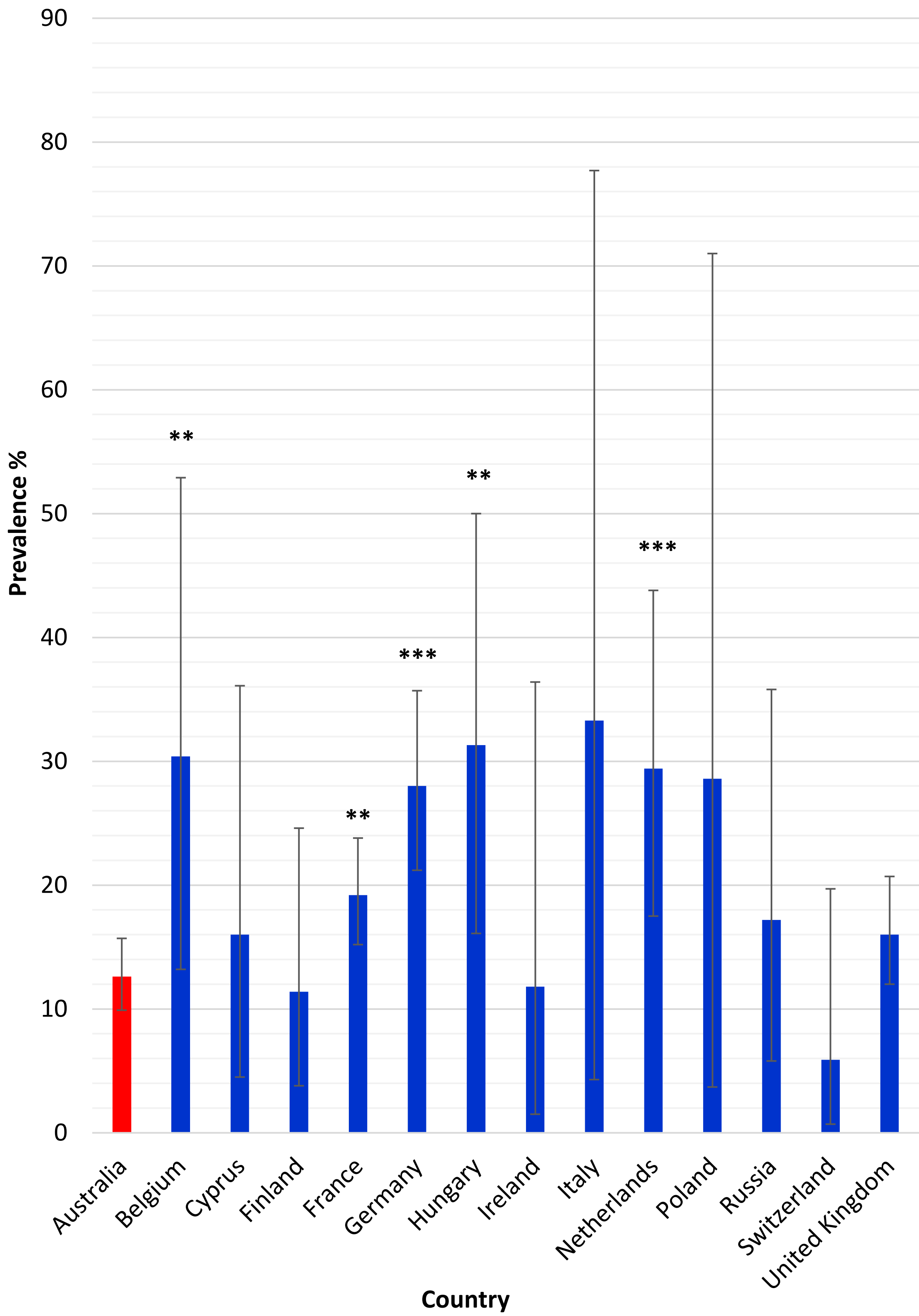

Figure 3.1: Reported prevalence and 95% CI for lumpy jaw in macropods housed in zoological institutions across Australia and Europe during the five years from 2011 to 2015. $^{**}p \leq 0.01$; $^{***}p \leq 0.001$ when compared to Australia.

Table 3.4: Odds ratios, 95% CI and Chi-square (Fisher's exact) *p*-value for the relationships between zoological institutions in European countries, and Australia, reporting cases of lumpy jaw in macropods. Australia is the comparative factor.

Country	**Odds ratio (95% CI)**	**Chi-square (Fisher's exact)**	***p*-value**
Belgium	3.04 (1.21 – 7.67)	6.11	0.01**
France	1.66 (1.14 - 2.40)	7.19	0.007**
Germany	2.71 (1.76 - 4.17)	21.58	<0.0001***
Hungary	3.16 (1.44 - 6.96)	8.97	0.003**
Netherlands	2.90 (1.51 - 5.57)	10.97	0.0009***

**$p \leq 0.01$; ***$p \leq 0.001$

Respondents of the survey sourced institutional lumpy jaw data from both zoo records and direct verbal reports from zoo staff. Of the 54 institutions that reported having one or more cases of lumpy jaw during the period covered by the survey, 63% (34/54) of these institutions reported sourcing their data only from zoo records; 13% (7/54) of institutions sourced their information directly from zoo staff and not zoo records; and 11.1% (6/54) used a combination of both zoo records and staff. The remaining 13% (7/54) institutions did not report the source of their lumpy jaw data.

3.4.3 Potential risk factor analysis

Species

All 54 (100%) of the responding institutions identified the species affected with lumpy jaw. Results indicated that 14 species of macropod housed in institutions across Australia and Europe developed lumpy jaw between 2011 and 2015. Of the three species that were housed in both the Australian and European regions, the red kangaroo had the greatest overall prevalence of the disease, at 23.1% (95% CI: 18.3 - 28.4, n = 286). Odds ratios confirmed there was no significant association between geographic region where the species were housed, and likelihood of lumpy jaw for the red kangaroo (OR 0.85, 95% CI: 0.41 - 1.76, n = 286, $p = 0.7$), the swamp wallaby

(OR 0.51, 95% CI: 0.07 - 3.51, p = 0.64 (Fisher's exact), n = 35) or the red-necked wallaby (OR 1.34, 95% CI: 0.73 - 2.47, p = 0.35, n = 765). Prevalence and 95% CI for species from both the Australian and European regions combined, and per region are reported in Table 3.5.

Table 3.5: Prevalence and 95% CI for lumpy jaw per macropod species from both the Australian and European regions and individually and combined (2011 - 2015).

Genus	Species common name	Australia n: Europe n	Combined prevalence % (95% CI)	Australian prevalence % (95% CI)	European prevalence % (95% CI)
Dendrolagus	Bennett's tree kangaroo	0:7	-	-	28.6% (3.7 - 71.0)
	Goodfellow's tree kangaroo	19:0	-	10.5 (1.3 - 33.1)	-
Macropus	Eastern grey kangaroo	4:0	-	25% (0.6 - 80.6)	-
	Parma wallaby	0:24	-	-	16.7% (4.7 - 37.4)
	Red kangaroo	53:233	23.1% (18.3 - 28.4)	20.8% (10.8 - 34.1)	23.6% (18.3 - 29.6)
	Red-necked*wallaby	61:704	20% (17.2 - 23.0)	24.6 % (14.5 - 37.3)	19.6% (16.7 - 22.7)
	Tammar wallaby	79:0	-	20.3% (12.0 - 30.8)	-
	Western brush wallaby	14:0	-	21.4% (4.7 - 50.8)	-
	Western grey kangaroo	0:69	-	-	10.1% (4.2 - 19.8)
Petrogale	Yellow-footed rock wallaby	203:0	-	4.9% (2.4 - 8.9)	-
Setonix	Quokka	74:0	-	5.4% (1.5 - 13.3)	-
Thylogale	Tasmanian pademelon	14:0	-	21.4% (4.7 - 50.8)	-
	Red-legged pademelon	1:0	-	100% (2.5- 100)	-
Wallabia	Swamp wallaby	19:16	14.3% (4.8 - 30.3)	10.5% (1.3 - 33.1)	18.8% (4.0 - 45.6)
	Unknown sp.	0:3	-	-	33.3% (0.8 - 90.6)

** Includes the Bennett's wallaby, a subspecies of the red-necked wallaby.*

Sex and age

The sex of macropods diagnosed with lumpy jaw over a five-year period was reported from 79.6% (43/54) responding institutions, for a total of 277 individuals. Collectively, across both regions, 36.5% (101/277) of individuals that were reported to have had lumpy jaw were male, 58.1% (161/277) were female and 5.4% (15/277) were of unknown sex. However, data were not provided for non-cases (the sex of macropods that did not experience a case of lumpy jaw), so odds ratios, and subsequent significant associations, could not be calculated. Regional differences for sex and age data are presented in Table 3.6.

A total of 39 institutions described the age of individuals affected by lumpy jaw; 94% (237/252) were reported as adults and 6% (15/252) as juveniles. Table 3.6 presents regional data for age categories for macropods diagnosed with lumpy jaw over a five-year period (2011-2015). Odds ratios showed that for the Australian region, adult macropods were 12.5 times more likely to be diagnosed with lumpy jaw than juveniles (OR 12.53, 95% CI: 0.74 - 212.32, *p* = 0.03, n = 252).

Table 3.6: Sex and age categories for macropods diagnosed with lumpy jaw in the five-year period 2011 – 2015, in relation to geographic region housed; Australia and Europe.

Region	Sex %			Age %	
	Male	Female	Unknown	Adult	Juvenile
Australia	28.8% (19/66)	62.1% (41/66)	9.1% (6/66)	100% (68/68)	0% (0/68)
Europe	38.9% (82/211)	56.9% (120/211)	4.3% (9/211)	91.8% (169/184)	8.2% (15/184)

3.4.4 Veterinary support

Frequency of veterinary support

The frequency of veterinary support was reported by 78.8% (63/80) institutions. Figure 3.2 indicates a greater daily presence of a veterinarian in institutions in the Australian region 75% (6/8) than in the European region 52.7% (29/55). Odds ratios found no significant association between frequency of veterinary presence and lumpy jaw in both the Australian and the European regions (OR 2.59, 95% CI: 0.84 – 7.96, *p* = 0.09, n = 63).

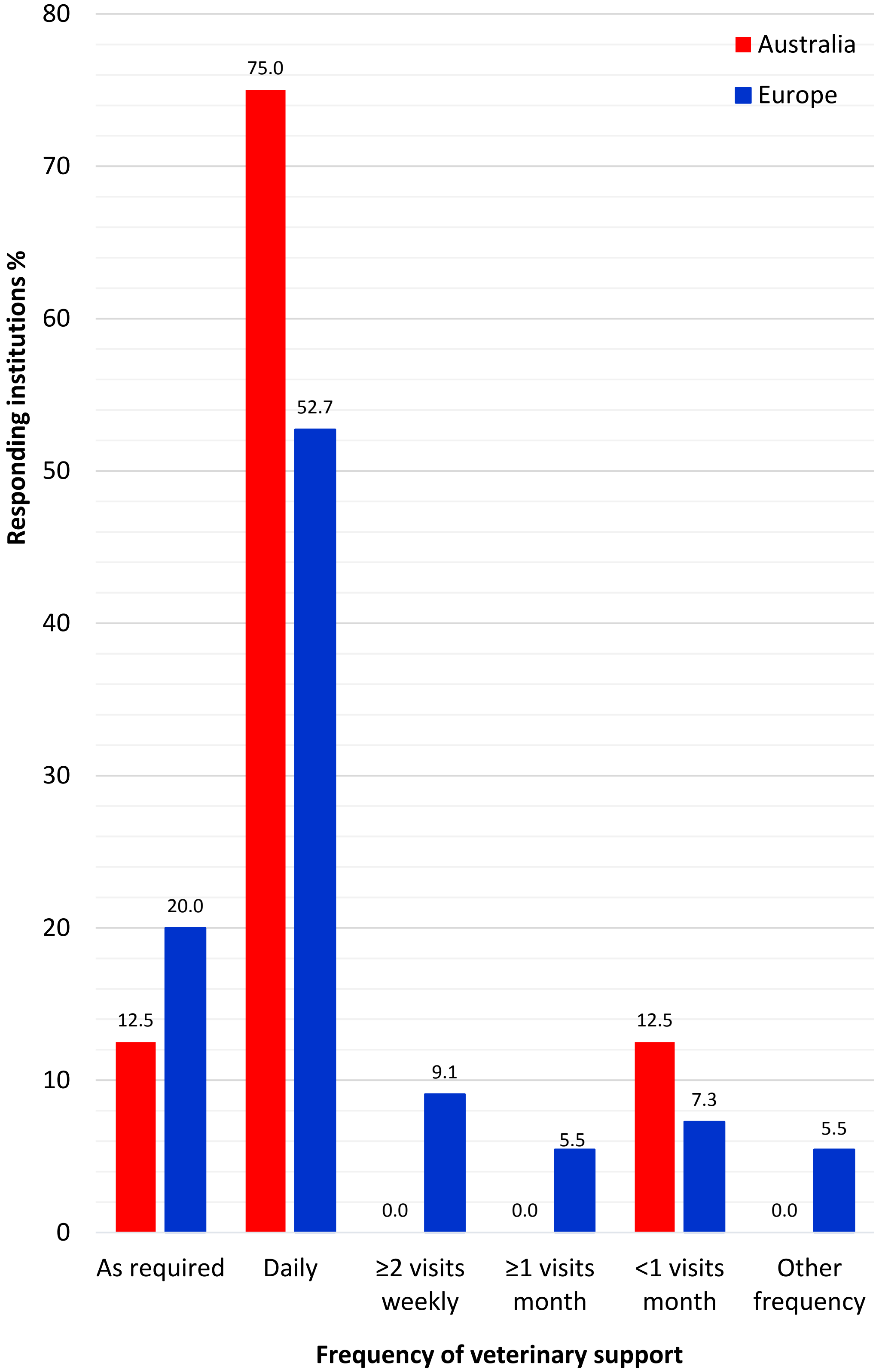

Figure 3.2: Frequency of veterinary support at Australian and European institutions housing macropods: response to a survey to determine prevalence and risk factors for lumpy jaw in captive macropods.

Health assessment

A total of 93.8% (75/80) responding institutions reported the frequency and method of routine health assessment of their macropods, of varying frequencies for each method undertaken. Results indicated that macropods in the Australian region most frequently had health assessments every 12 – 24 months, using each method available (Figure 3.3 a, b, c). All methods of health assessments in the European region most frequently occurred as required or opportunistically (Figure 3.3 a, b, c).

Oral/dental examinations were undertaken by 82.5% (66/80) of responding institutions during a health assessment, whereas 81.1% (43/53) reported the use of radiography of the teeth and jaw when undergoing a health assessment.

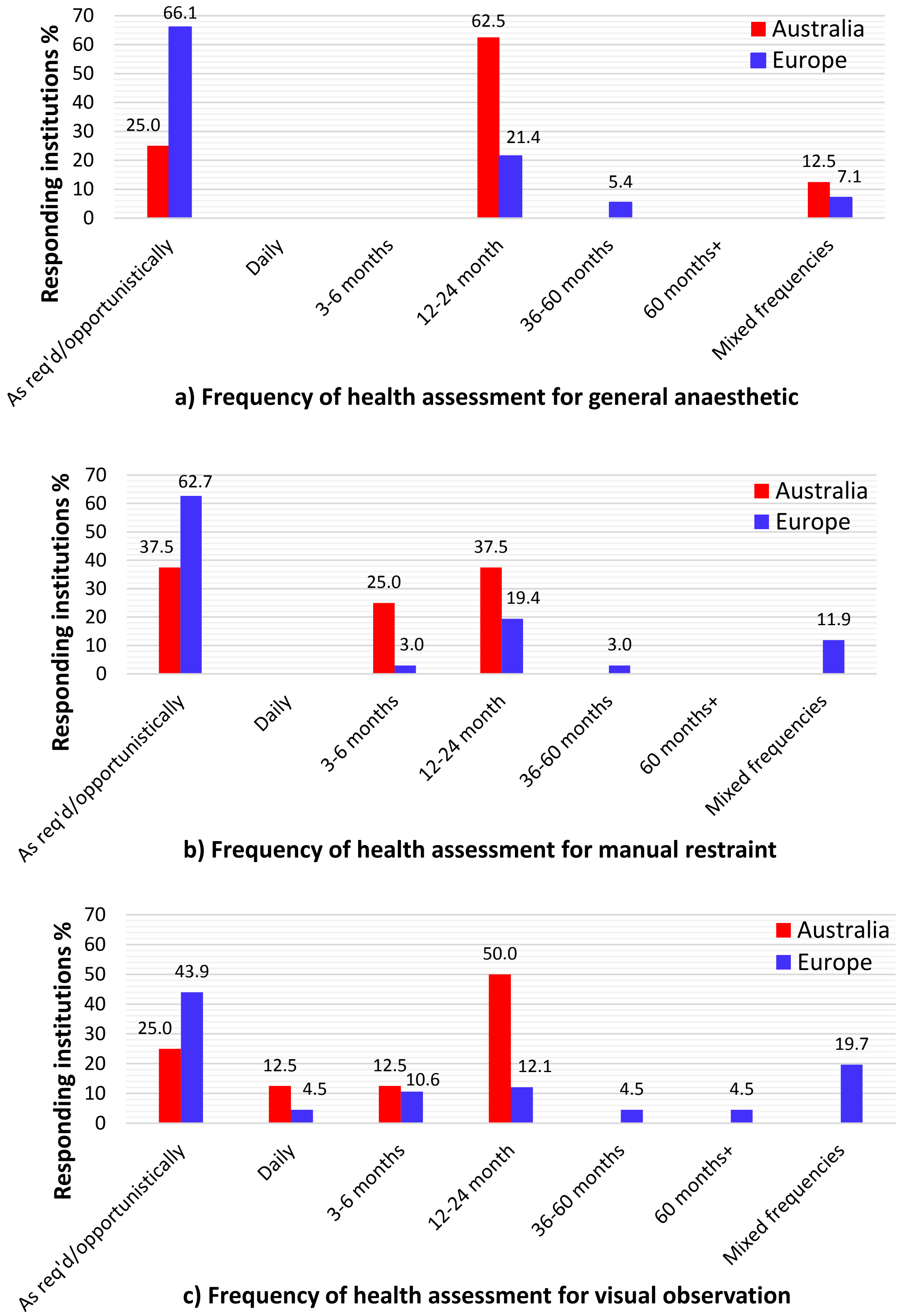

Figure 3.3: Frequency and method of health assessments on macropods undertaken in institutions across Australia and Europe: a) under general anaesthetic, b) under manual restraint, c) using visual observation.

3.4.5 Diagnosis

Initial detection

Results showed that the majority of responding institutions, 87.7% (50/57), used keeper observation of behavioural or clinical signs to detect cases of lumpy jaw. Routine visual health assessment was used to detect cases of lumpy jaw at 5.3% (3/57) of institutions, and 1.8% (1/57) reported the detection of cases during routine health assessment under general anaesthetic. A total of 3.5% (2/57) of institutions reported using a mixed methods approach to detect case of lumpy jaw.

Diagnostic method

Institutions were asked about the diagnostic methods used in the detection of lumpy jaw in their macropods. The leading diagnostic method used was the observation of clinical signs, with 82.5% (47/57) of responding institutions reporting the use of this method (Table 3.7). Microbial culture was used to a greater extent in the Australia region than in the European region (Australia 66.7%, 4/6; Europe 60.8%, 31/51). Radiography was also carried out more frequently in the Australian region (Australia 83.3%, 5/6; Europe 64.7%, 33/51). Although microbial culture was used less frequently than the other method options, it was still reported as being undertaken in 61.4% (35/57) of responding institutions. A single institution reported that they used 'other' methods to diagnose lumpy jaw, however no further information was provided.

Table 3.7: Method and frequency of diagnosis of lumpy jaw in macropods housed in institutions across the Australian and European region: response to a survey to determine prevalence and risk factors for lumpy jaw in captive macropods.

Diagnostic method	**Combined region % (x/n)**	**Australian region % (x/n)**	**European region % (x/n)**
Clinical signs	82.5% (47/57)	100% (6/6)	80.4% (41/51)
Microbial culture	61.4% (35/57)	66.7% (4/6)	60.8% (31/51)
Radiography	66.7% (38/57)	83.3% (5/6)	64.7% (33/51)

Bacterial culture

Bacterial culture was reported to have been used by 56.1% (32/57) responding institutions during the diagnosis of lumpy jaw. These institutions provided data relating to the identity of bacterial species cultured for cases of lumpy jaw (Table 3.8). *Fusobacterium necrophorum* was the most frequently cultured species in the European region; whereas *Pseudomonas aeruginosa* was the most frequently cultured species in the Australian region. Other species cultured that were reported by institutions are provided in Appendix E.

Table 3.8: Reported bacterial species cultured in cases of lumpy jaw by Australian and European zoological institutions responding to a survey to determine prevalence and potential risk factors for lumpy jaw in captive macropods (% = the number responses/total intuitions responding). Some institutions reported more than one bacterial species.

Bacterial species	**Australian region % (x/4)**	**European region% (x/28)**
Actinobacillus muris	-	3.6% (1)
Bacteroides nodosus	-	3.6% (1)
Bacteroides pyogenes	-	7.1% (2)
Bacteroides ruminicola	-	7.1% (2)
Enterococcus faecalis	-	21.4% (6)
Escherichia coli	-	25% (7)
Fusobacterium necrophorum	25% (1)	28.6% (8)
Neisseria weaveri	-	3.6% (1)
Pseudomonas aeruginosa	50% (2)	17.9% (5)
Peptostreptococcus anaerobius	-	3.6% (1)
Prevotella heparinolytica	-	3.6% (1)
Streptococcus uberis	-	7.1% (2)
Other (Appendix E)	50% (2)	32.1% (9)

3.4.6 Treatment

Treatment options

The majority of responding institutions that experienced lumpy jaw provided data relating to the treatment delivered (96.5%, 55/57). The use of systemic antibiotic therapy was the most frequently reported treatment, with 81.8% (45/57) of institutions reporting its use. Systemic antibiotics were used to a lesser extent in the Australian region (60%, 3/5) than in the European region (86%, 43/50). A greater percentage of institutions in the Australian region reported the use of tooth extraction (80%, 4/5) and debridement (80%, 4/5) than those in the European region (60%, 30/50 and 42%, 21/50 respectively). Euthanasia was reported as a treatment option by 100% (5/5) of Australian institutions and 62% (36/50) of those in Europe. 'Other' treatment options reported by respondents were not provided. Results for the range of treatments undertaken for lumpy jaw are presented in Table 3.9.

Table 3.9: Methods used in the treatment for lumpy jaw in captive macropods in zoological institutions across the Australian and European regions: response to a survey to determine prevalence and risk factors for lumpy jaw in captive macropods.

Treatment	**Combined regions % (x/n)**	**Australian region % (x/n)**	**European region % (x/n)**
Antibiotic impregnated beads	40% (22/55)	20% (1/5)	42% (21/50)
Apicoectomy	-	-	24% (12/50)
Debridement	45.5% (25/55)	80% (4/5)	42% (21/50)
Euthanasia	74.5% (41/55)	100% (5/5)	62% (36/50)
Laser therapy	-	-	18% (9/50)
Nutritional supplementation	-	-	42% (21/50)
Oral varnishes/gels e.g. chlorhexidine	36.4% (20/55)	60% (3/5)	38% (19/50)
Systemic antibiotic therapy	81.8% (45/55)	60% (3/5)	86% (43/50)
Tooth extraction	70.9% (39/55)	80% (4/5)	60% (30/50)
Other[a]	-	-	8% (4/50)

[a]Respondents did not provide information relating to 'other' treatments used.

Antibiotics

Of the 57 responding institutions that reported cases of lumpy jaw, 77.2% (44/57) provided data on the antibiotics used in the treatment of the condition (Figure 3.4). The use of more than one antibiotic was reported by 68.2% (30/44) institutions. Two institutions indicated they did not use antibiotics (2/44). The leading group of antibiotics used collectively was the penicillins, 61.4% (27/44). The penicillins were also the leading antibiotic of choice in both the Australian and European regions (80%, 4/5 and 59%, 23/39 respectively).

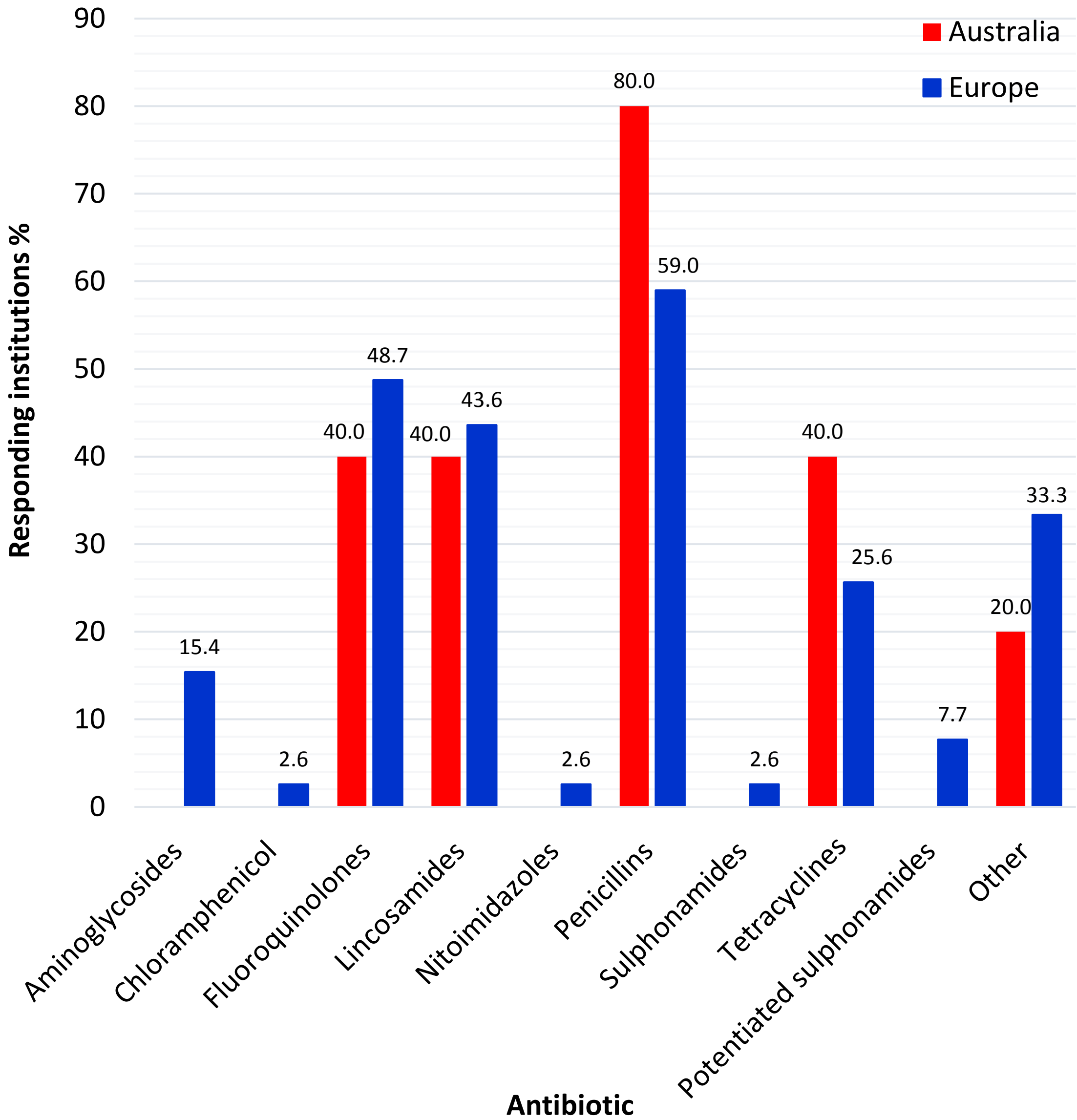

Figure 3.4: Antibiotics used in the treatment of lumpy jaw in macropods housed in Australian and European institutions (2011 – 2015): response to a survey to determine prevalence and risk factors for lumpy jaw in captive macropods.

Vaccination

A total of 98.2% (56/57) of responding institutions that reported cases of lumpy jaw provided a response to the survey question about use of vaccinations for lumpy jaw. Eight (14.3%) institutions reported the use of vaccinations for lumpy jaw, although only two provided product details (Footvax®, Schering-Plough Animal Health Limited, Upper Hutt, New Zealand; Bestvac Rind Mastitis®, IDT Biologika GmbH, Dessau-Rosslau), with 85.7% (48/56) of institutions reporting having not used them.

Outcome

Of the responding institutions that reported cases of lumpy jaw in the last five years, 77.2% (44/57) provided details of the outcome (death) of those affected with the disease. A total of 82.8% (178/215) macropods died as a result of lumpy jaw (unassisted n = 50; euthanased n = 128) (Table 3.10).

Odds ratios determined that once diagnosed with lumpy jaw, a macropod was no more likely to die (including both unassisted deaths and euthanasia) if originating from an institution in either the Australian or European region (OR 1.66, 95% CI: 0.76 - 3.59, $p = 0.2$, n = 200).

Once diagnosed with lumpy jaw, the likelihood of being euthanased, as opposed to having an unassisted death, was higher in the Australian region than in Europe (OR 3.39, 95% CI: 1.18 - 8.75, $p = 0.01$, n = 183).

Table 3.10: Fate of macropods diagnosed with lumpy jaw at Australian and European institutions over a five-year period (2011 - 2015): response to a survey to determine prevalence and risk factors for lumpy jaw in captive macropods.

Type of death	**Combined regions %** **(x/n)**	**Australian region %** **(x/n)**	**European region %** **(x/n)**
Unassisted death	23.3% (50/215)	9.6% (5/52)	27.6% (45/163)
Euthanasia	59.5% (128/215)	67.3% (35/52)	57.1% (93/163)
Total deaths (unassisted/euthanasia combined)	82.8% (178/215)	76.9% (40/52)	84.7% (138/163)
Presumed survived	17.2% (37/215)	23.1% (12/52)	15.3.% (25/163)

3.4.7 Housing and husbandry

Enclosure size

A total of 85% (68/80) institutions provided enclosure size relating to 135 individual enclosures used for macropods. Enclosure size ranged from 17 m^2 to 100,000 m^2 (median 831.5 m^2); median enclosures in the Australian region were larger than those in the European region (Australia 1137 m^2, Europe 750 m^2). Enclosure area per macropod across both regions ranged from 1.9 m^2 per individual to 14,285.7 m^2, with a median of 128.5 m^2. Macropods in the Australian region had nearly twice the enclosure area per individual compared to macropods in the European region (Australia median 220.9 m^2, European median 113.3 m^2).

Enclosure substrate

A total of 98.8% (79/80) responding institutions provided information on enclosure substrate for 162 macropod enclosures. The most frequently reported substrate in the Australian region was mulch, with 35.9% of macropod enclosures containing mulch as the primary substrate. Grass was the most commonly reported substrate in macropod enclosures in the European region (55.3%) (Figure 3.5).

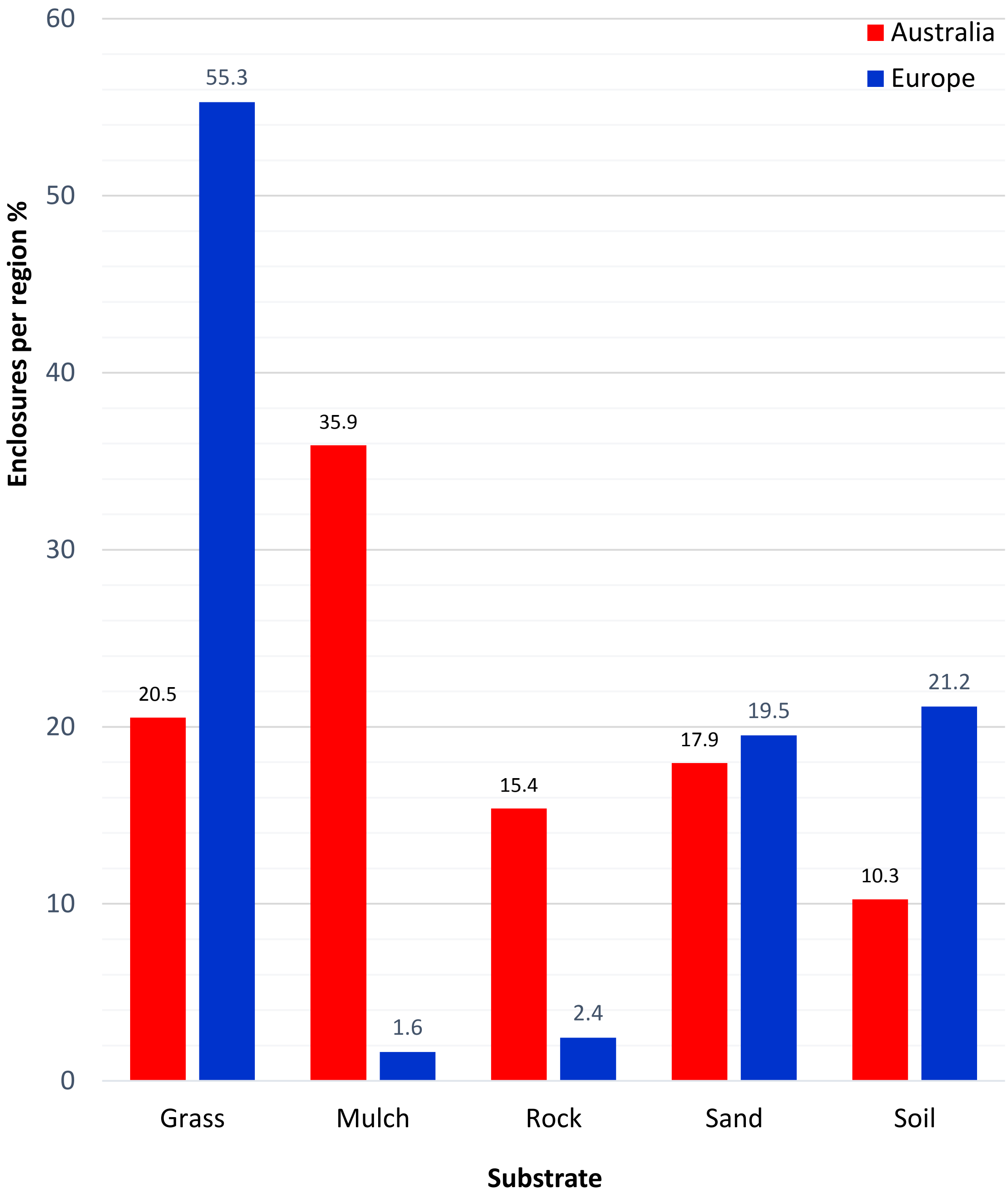

Figure 3.5: Regional enclosure substrate used in macropod exhibits across the Australian (n = 39) and European region (n = 162): response to a survey to determine prevalence and potential risk factors for lumpy jaw in captive macropods.

Enclosure type

All 80 (100%) responding institutions provided enclosure type data for 165 individual enclosures. Combined data for both the Australian and European regions revealed macropods were most commonly housed in single species (SS) enclosures (53/165). SS enclosures were also the leading method used for the display of macropods at a regional level (Australia 11/39; Europe 42/126) (Figure 3.6).

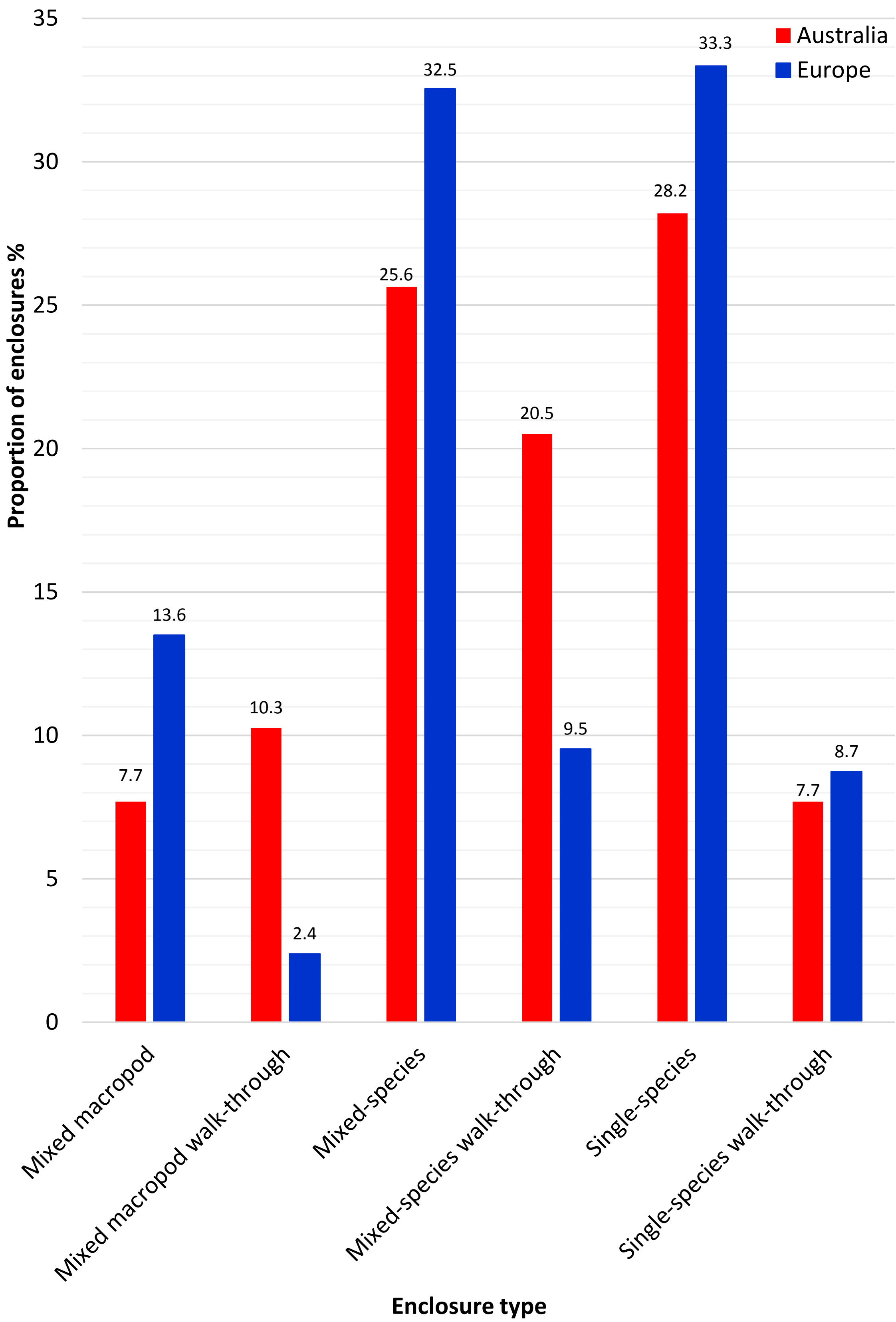

Figure 3.6: Regional enclosure type used for macropod exhibits across the Australian (n = 39) and European region (n = 165): response to a survey to determine prevalence and risk factors for lumpy jaw in captive macropods.

Dietary content

A total of 92.5% (74/80) of responding institutions provided dietary data, resulting in 131 feeding records for 19 macropod species.

Across the Australian and European regions, the most commonly fed dietary items were pellets and hay/grass, with 95.4% (125/131) feeding records from responding institutions reporting these items as being fed to their macropods. Fruit was observed more commonly in the feeding records in European institutions. The feeding of bread was reported in 15.3% (20/131) of feeding records, all of which were from the European region (equating to 19.2% of European feeding records) (Figure 3.7). There were no significant associations observed between the odds ratios of lumpy jaw and potential risk factors relating to diet.

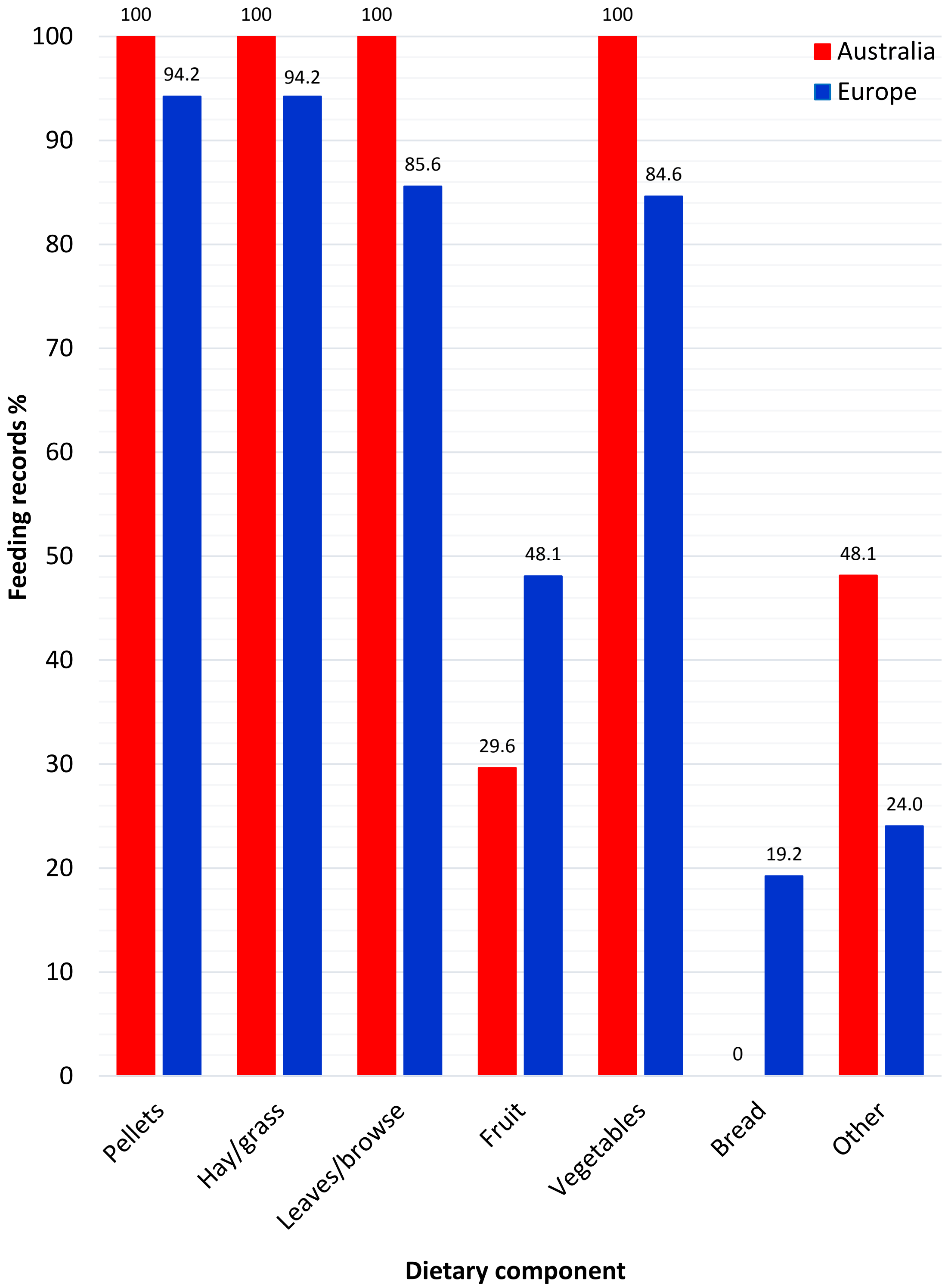

Figure 3.7: Percentage of macropod feeding records from zoological institutions across Australia (n = 27 diet records) and Europe (n = 104 feeding records), reporting the various components of macropod diet: response to a survey to determine prevalence and risk factors for lumpy jaw in captive macropods. 'Other' includes once or more of – oats, barley flakes, dried corn, rolled oats, pumpkin seeds, sunflower seeds, wheat porridge of buckwheat and wheat, cereal sprouts, peanuts.

Diet delivery methods

The section of the survey relating to the method of delivery of dietary components was completed by 68/80 (85%) responding institutions, resulting in 70 records of feeding practices for the various components of the macropod diet. Ground feeding was the leading method of choice for all dietary components (Table 3.11). Odds ratios showed that macropods in this study were no more likely to have lumpy jaw if fed off or on the ground, irrespective of the dietary component.

Table 3.11: Components of the macropod diet and respective feeding methods as reported by zoological institutions across Australia and Europe: response to a survey to determine prevalence and potential risk factors for lumpy jaw in captive macropods.

Dietary component	Scatter	Ground	Raised	Hand	Mixed	Other[a]
Pellet	2/52	32/52	9/52	-	8/52	1/52
Hay/grass	10/59	13/59	20/59	-	12/59	4/59
Leaves/browse	26/60	21/60	2/60	-	6/60	5/60
Fruit	1/29	20/29	4/29	1/29	2/29	1/29
Vegetable	2/48	31/48	7/48	-	6/48	2/48
Bread	2/11	3/11	1/11	2/11	2/11	1/11
Other[b]	-	3/9	5/9	1/9	-	-

N.B. *Scatter* – scatter feeding and the feeding from bowl/trough placed on the ground enabling faecal contamination; *Raised* – trough/bowl/net or other method of raising item off the ground; *Hand* – fed directly from the hand of a keeper or visitor (unclear as to which); *Mixed* – responding institution reported more than one method used. [a]*Other* – diet fed using individual bowl or by other means not recorded. [b]*Other dietary component*– dried corn kernels, peanuts.

Enclosure cleaning frequency

A total of 88.8% (71/80) of institutions provided data on the cleaning frequency of macropod enclosures. Collectively (Australian and European regions data combined), there was no significant association between cleaning frequency of water containers and the likelihood of lumpy jaw, where water containers were cleaned every 1 - 2 weeks compared to cleaning on a daily basis (OR 9.33, 95% CI: 0.97 - 89.42, p = 0.08, n = 65). Although small sample sizes prohibited the calculation of OR for the Australian region, a non-significant result was reported for the European region (OR 7.56, 95% CI: 0.78 - 72.74, p = 0.07, n = 52). Notably, no association was observed between reports of 'never' cleaning (each area of) macropod enclosures, and cases of lumpy jaw. 'Daily' cleaning of (all areas of) macropod enclosures was most frequently reported by responding institutions (Figure 3.8).

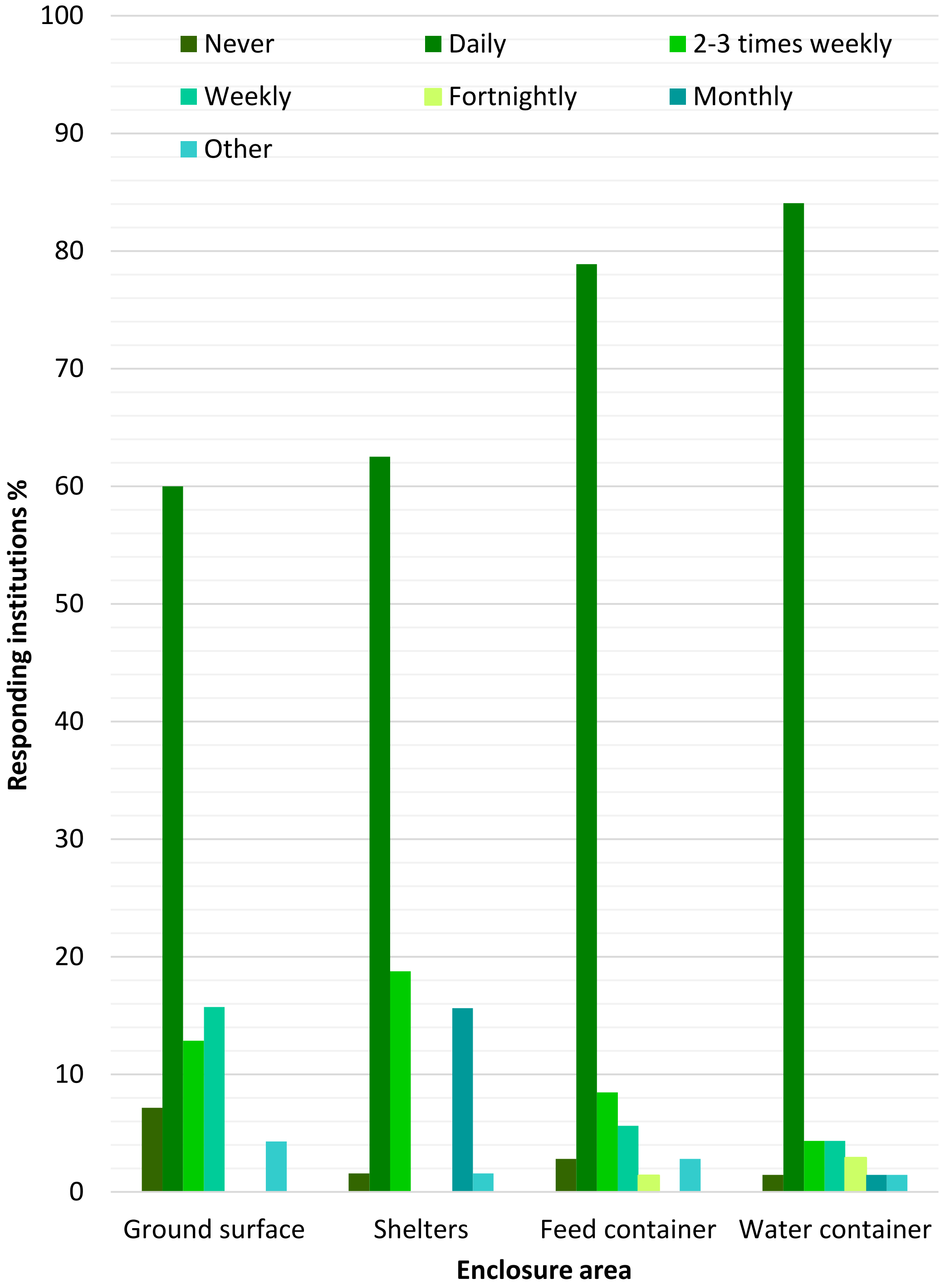

Figure 3.8: Percentage of responding institutions reporting on cleaning frequency of macropod enclosures in zoological institutions across the Australian and European regions combined: response to a survey to determine prevalence and potential risk factors for lumpy jaw in captive macropods.

Enclosure cleaning method

With one exception (feed containers), the most frequently reported cleaning method for (all areas of) macropod enclosures was 'mechanical' methodology, where chemical agents were not used (Figure 3.9). For all enclosure areas, there was no association found between the method of cleaning macropod enclosures and cases of lumpy jaw, for both regions.

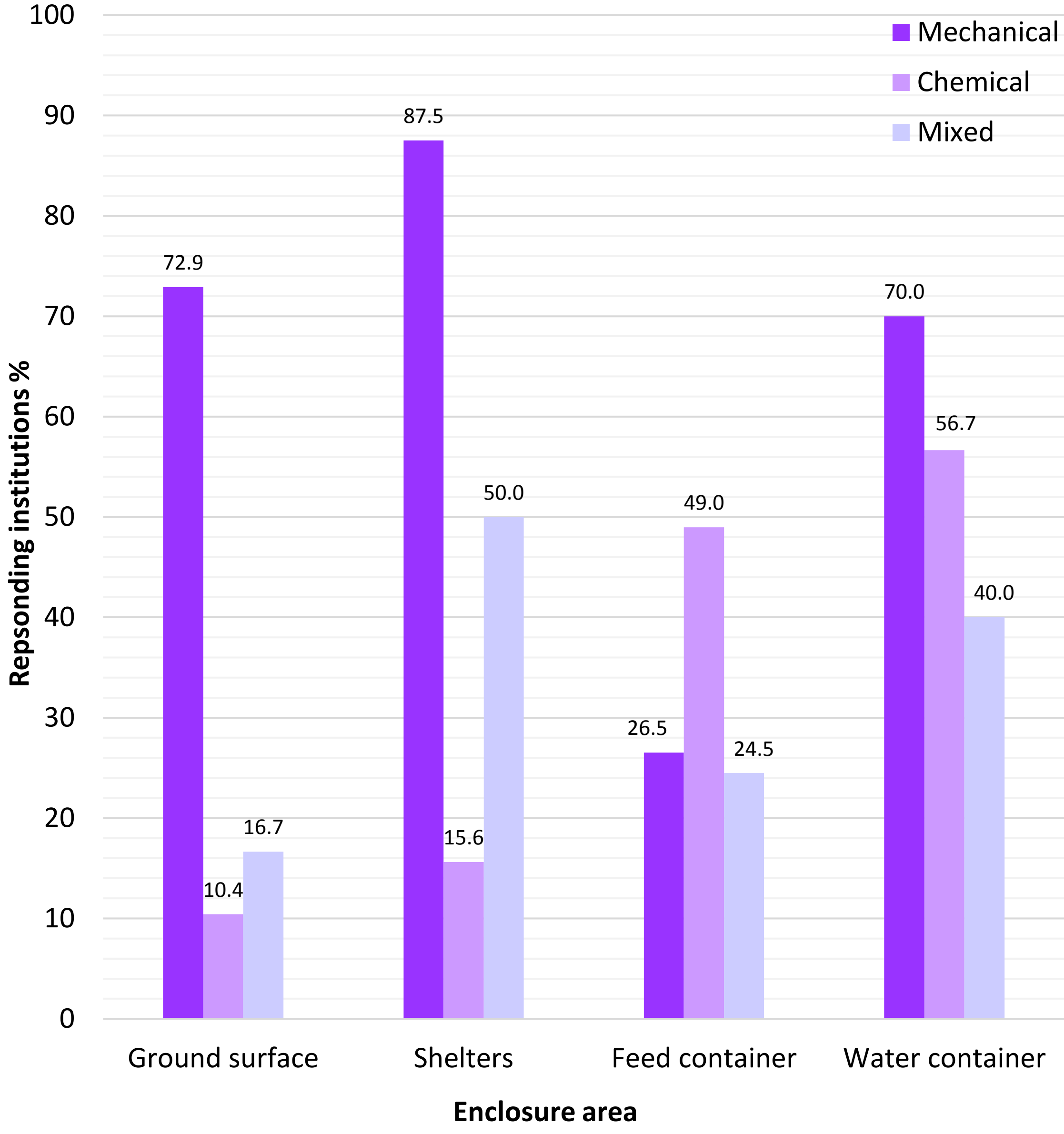

Figure 3.9: Percentage of combined Australian and European responding institutions reporting cleaning methods used in various areas of macropod enclosures. Cleaning method: *Chemical* – use of detergent, disinfectant or another cleaning agent. *Mechanical* – use of tools and equipment. *Mixed* – use of both agents and tools.

Staff biosecurity

A total of 90% (72/80) of institutions provided data relating to personnel biosecurity measures involving footwear, and 87.5% (70/80) reported results for hand washing. Two institutions were removed from further calculations due to conflicting data being reported in this section (several selections had been made when only one was required).

Institutions that did not undertake biosecurity of footwear were 4.6 times more likely to have cases of lumpy jaw than those that routinely undertook footwear cleaning procedures (OR 4.57, 95% CI: 1.45 - 14.42, p = 0.01, n = 72). No significant association was found between the washing of hands and cases of lumpy jaw in macropods.

Tool biosecurity

A total of 71.3% (57/80) responding institutions reported the routine cleaning of tools after each use, and 73.8% (59/80) institutions reported the cleaning of tools between enclosures.

Institutions were 13.1 times more likely to have cases of lumpy jaw if they did not clean tools after each use (OR 13.09, 95% CI: 1.58 - 108.42, p = 0.005, n = 57), and 6.8 times more likely to have cases of lumpy jaw if they did not clean tools in between enclosures (OR 6.82, 95% CI: 1.38 - 33.6, p = 0.02, n = 59).

3.5 Discussion

Our study describes, for the first time, a range of regional and country level data regarding lumpy jaw in macropods in zoological institutions across two regions, Australia and Europe, including potential risk factors for the disease. Over 70% (71.3%) of survey respondents, from across 16 countries, reported cases of lumpy jaw in their macropods in the last five years (2011 – 2015); resulting in a combined period prevalence of 17.4%. This figure suggests lumpy jaw remains an important cause of morbidity and mortality for zoos housing kangaroos and wallabies. A significantly greater period prevalence of 19.9% was reported in the European region compared to 12.6% reported in the Australian region. A diagnosis of lumpy jaw resulted in the death of 82.8% of macropods, and those in the Australian region were 3.4 times more likely to be euthanased compared to European macropods (p = 0.01). Adult macropods were at significantly greater risk of being diagnosed with lumpy jaw than juvenile macropods, although this association was only determined for macropods housed in the Australian region. In addition, reduced biosecurity measures undertaken by zoo personnel, including less frequent cleaning of tools and footwear, are associated with increased risk of lumpy jaw in captive macropods; providing empirical evidence to support hypotheses that these housing and husbandry approaches, involving cleaning and biosecurity, are useful strategies to manage potential risk factors for lumpy jaw. This survey has substantially increased our regional and global understanding of risk factors associated with the development of lumpy jaw, providing hypotheses for further research, and an evidence-base for the improved management of captive macropods to minimise cases of this often-fatal condition.

3.5.1 Prevalence

The leading aim of this survey was to determine the prevalence of lumpy jaw in captive macropods from two regions where macropods are popular exhibits, Australia and Europe. Results found that macropods housed in the European region were nearly two times more likely to have lumpy jaw than Australian macropods (p = 0.0003). Prevalence of lumpy jaw varied considerably between the individual countries, and was detected in countries not previously identified in the literature (Austria, Belgium, Cyprus, Finland, Ireland, Netherlands, Poland, Russia and Switzerland). Italy reported the highest prevalence at 33.3%, although its interpretation is challenging due to the small sample size. Switzerland reported the lowest prevalence, at 5.9%, a result that when examined across European countries, was significantly lower than that reported for Germany (p = 0.004). Five European countries had significantly higher prevalence than Australia (Belgium, France, Germany, Hungary and the Netherlands). These results present the possibility that geographic and potentially any associated climatic factors in these European countries, may increase the likelihood of lumpy jaw occurring in captive macropods. However, we cannot rule out confounding factors associated with broader approaches to macropod management, which may differ between the Australian and European regions.

The cooler, wetter climate found in the European region (Kottek et al., 2006), may facilitate the survival of pathogenic bacteria within the enclosure substrate (Oliphant et al., 1984); this is an area that warrants further investigation. Most macropods are endemic to Australia and Papua New Guinea (Sillmann et al., 2011; Lewis et al., 2017), and the exposure to the typically cooler and wetter European region may act as a stressor. An animal's inability to regulate their environmental temperature, or migrate to a temperate area, can act as a stressor (Morgan & Tromborg, 2007), and response to the European climate could subsequently result in immunosuppression and the development of disease (Dohms & Metz, 1991; Biondi & Zannino, 1997). As a species more suited to the temperate regions of Australia, and can be found in the alpine regions of Tasmania, it could be expected that the red-necked wallaby would perform well in a European climate. However, our results indicate that nearly one in five red-necked wallabies (19.6%) succumbed to the disease in Europe. For swamp wallabies, it was notable that the prevalence of lumpy jaw reported in Europe of 18.8%, was higher than that reported for the same species in Australia 10.5%, although this result was not statistically significant (p = 0.6). Overall, our results provide support for Kido et al.'s (2013) suggestion that a high prevalence of lumpy jaw is associated with a colder, wetter climate; however, other differences between macropod species, besides the ability to adapt to the climate, should also be considered, including management practices, diagnostics and veterinary presence.

3.5.2 Risk factor analysis

Several potential risk factors have previously been associated with the development of clinical lumpy jaw, including age and its relationship with molar progression (Sanson, 1989; Clarke, 2003; Lentle et al., 2003), aspects of housing and husbandry (Burton, 1981; Vogelnest & Portas, 2008), and species (Sanson, 1989; Vogelnest & Portas, 2008). Survey respondents reported that 19 macropod species were housed across both the Australian and European regions, of which 14 species were reported to have been diagnosed with lumpy jaw. Previous studies have reported prevalence rate by species (Kido et al., 2013), or as the result of detection of lumpy jaw at necropsy (Vogelnest & Portas, 2008); leading to a potential misrepresentation of the prevalence of the disease. The results from our study contribute to the literature by providing the prevalence for 14 species of macropod recently/currently housed in zoos across two geographical regions simultaneously. These data provide a reliable representation of the prevalence of lumpy jaw in captive macropods for the locations and period of study, to complement the prevalence reported for individual species or during necropsies in earlier studies (Vogelnest & Portas, 2008; Kido et al., 2013).

Species

Results from the present study demonstrate variation in reported prevalence of lumpy jaw between macropod species; however, differences in prevalence were also reported within species, which may be the result of regional or institutional differences, or the effect of sample size. There was a single red-legged pademelon (*Thylogale stigmatica*) in the study, which was reported as having lumpy jaw, however, it is difficult to interpret these findings given the small sample size. The yellow-footed rock wallaby (*Petrogale xanthopus*) was found to have a significantly lower prevalence of lumpy jaw compared to the red kangaroo, the red-necked wallaby and the tammar wallaby (*Macropus eugenii*). In addition, the quokka also had a significantly lower prevalence of lumpy jaw than the red-necked wallaby. These findings could be associated with anatomical differences in dentition between these species, or as a response to the climate in the country where they were housed.

Prevalence of lumpy jaw in particular species may be the result of species adaptability to the climate in which they were housed. The red kangaroo, evolved to live in the hot, arid centre of the Australian outback, may not be best suited to the cooler, wetter European climate. However, our research found no significant difference between the prevalence of lumpy jaw in red kangaroos housed in the Australian region and those housed in Europe. Higher prevalence of lumpy jaw has been reported for this species in the Australian region, with Vogelnest and Portas (2008) reporting a prevalence of 29.6% (95% CI: 18.0 - 43.6) at one institution, and Burton (1981) reporting a prevalence of 58.3% (95% CI: unknown) at another. Both these figures are based on institutional findings over discrete and historic time periods, but the prevalence we now present for the Australian region is not statistically different from those previously presented ($p = 0.1$), indicating that improvements in efforts to control the disease in Australian institutions are still required. Further institutional investigations may identify key factors responsible for the lower prevalence in our data presented for the red kangaroo.

Sex

One of the suggested risk factors for lumpy jaw is stress (Butler & Burton, 1980; Ketz, 1997; Vogelnest & Portas, 2008). Stress in captivity may be related to, among other things, inappropriate social groupings, such as an inappropriate mix of sex or age classes (Morgan & Tromborg, 2007; Rees, 2011). Appropriate sex ratios for captive macropods vary between species; and in this study, sex could not be examined as a potential risk factor for lumpy jaw due to incomplete data within the survey. Although the survey data revealed that a greater number of females were affected by the disease than males, we were not able to determine the proportions of females and males affected relative to the total number of individuals of each sex. This was due to a lack of data on the total number of individuals by sex, for each species and institution.

Age

Our study was, however, able to indicate that age appears to be a risk factor for lumpy jaw. Survey data showed that adult macropods were 12.5 times more likely to be affected with the condition than juveniles ($p = 0.03$). Ageing leads to a number of changes to the dental arcade, such as reduced salivary flow, which can lead to gingivitis and periodontitis (Glatt et al., 2008). Both gingivitis and periodontitis are precursors of lumpy jaw (Clarke, 2003; Antiabong et al., 2013b) and form part of the continuum of this disease (McLelland, 2019). Molar progression, another proposed risk factor for lumpy jaw, also occurs with age (Kirkpatrick, 1964; Dudzinski et al., 1977; Clarke, 2003; Kido et al., 2018). Whilst some previous authors have suggested that age-related molar progression is one of the main drivers of disease (Finnie, 1976; Arundel et al., 1977; Miller et al., 1978), a study of lumpy jaw in wild macropods, reported to the contrary (Borland et al., 2012). Prevalence of lumpy jaw in juveniles could be expected to be lower than in adults for a number of reasons, such as reduced exposure of pouch young to potential risk factors including housing type, substrate and diet. In addition, reported prevalence may be lower for juveniles, in part due to a lower likelihood of detecting disease in juveniles than adults during physical examinations, due to the small gape of juvenile macropods. Additionally, prevalence may also be reduced in juveniles due to exposure to the potentially protective factors of the microbiome from the maternal saliva whilst in the pouch, providing some immunity against pathogenic bacteria (Chhour et al., 2010), including pathogens associated with lumpy jaw. Despite the above, our survey data did show that a number of juveniles were diagnosed with the disease (n = 15) indicating that some younger individuals are 'at risk'. Guidance for the classification between the age categories (adult and juvenile), was not provided with the survey. The age at which a macropod is considered an adult is based on sexual maturity, and varies between species (Jackson, 2003). The lack of clarification relating to age categorisation may have affected the results. Future studies would benefit from the provision of a clear definition of the age categories, potentially including information relating to the age of sexual maturity per species, and whether the definition of juvenile includes stages of pouch young.

3.5.3 Veterinary support

The overall health and welfare of captive macropods, including the management of diseases such as lumpy jaw, is the responsibility of a team of zoo personnel, with ultimate responsibility lying with the veterinarian. Zoos use a combination of both keeper observation and veterinary knowledge to detect signs of ill health in their mobs (Hill & Broom, 2009). The majority of institutions surveyed in our study (66%) reported that initial detection of lumpy jaw was most frequently based on the observation of behavioural and clinical signs by keeping staff. Behavioural signs of lumpy jaw include pawing at the mouth, inappetence and difficulty masticating (Hartley & Sanderson, 2003; Vogelnest & Portas, 2008), and should be detected by an experienced keeper. Clinical signs such as swelling to the mandibular or maxillary region and hyperptyalism are aspects of progressive disease, and although discernible by keepers, confirmatory diagnosis by a veterinarian is still required. Veterinary examination is essential to detect the primary clinical signs of lumpy jaw, such as necrosis of the gingival mucosa (Antiabong et al., 2013a); this is most commonly carried out under general anaesthesia. Detailed examination of the mouth and jaw, whilst the macropod is chemically immobilised, will facilitate early detection of primary signs of lumpy jaw, and enables the subsequent initiation of appropriate treatment for the disease. Both early detection and treatment play a vital role in the expected outcome of a case of lumpy jaw (Lewis et al., 1989; Hartley & Sanderson, 2003; Jackson, 2003; Vogelnest & Portas, 2008), however this may be affected by the frequency and regularity of macropod health assessments. The early detection of lumpy jaw can be identified through regular health assessments, yet in the majority of responding institutions (60.1%), health assessments were carried out only ‘as required’, rather than ‘routinely’. This may be due to the requirement for chemical immobilisation to facilitate a thorough oral examination, which may not be practical, or feasible, in some institutions, due to lack of veterinary staff, resources or finances. The increased risk to macropod health (e.g. exertional myopathy) and the safety of zoo staff is a justifiable reason for infrequently undertaking veterinary examination of macropods.

Lumpy jaw in captive macropods may be detected during health assessments undertaken by the zoo veterinarian, however not all institutions have regular access to a veterinarian and the diagnostic resources to enable detailed health assessments that facilitate early detection. Health assessments were conducted in various ways, and most were carried out using visual observation or manual restraint, rather than under general anaesthetic. The capability of detecting early stages of lumpy jaw is limited unless a thorough examination of the oral cavity is undertaken, preferably under general anaesthetic (D. McLelland, personal communication, 16th August, 2016). Institutions where health assessments were carried out under general anaesthesia underwent a greater range of assessments more suited to the detection of lumpy jaw, such as oral/dental examination, and radiography of teeth and jaw. The institutions that had a regular veterinary presence may have had the ability to undertake detailed health assessments on anaesthetised macropods, thus enabling the detection of lumpy jaw. Our results show that where a veterinarian was present on a daily basis, as was found more frequently in the Australian region, fewer cases of lumpy jaw were detected; and notably, this same region had a relatively lower prevalence of the disease. Over half of all respondents from the European region (52.7%) reported having a veterinarian present on a daily basis; suggesting that the remaining proportion of European institutions may have been relying upon the knowledge and observations of keeping staff to detect signs of disease, rather than systematic health assessments carried out by a veterinarian. In contrast, and as shown in this study's survey, keepers were more likely to only detect chronic cases of lumpy jaw; which can be readily identified by visual observation of bony protrusions to the jaw. Delayed detection of the disease, for example in the absence of regular veterinary assessment, is less than ideal given that chronic cases of lumpy jaw are particularly challenging to treat (Vogelnest & Portas, 2008).

3.5.4 Diagnosis of lumpy jaw

Bacterial culture

Bacterial species previously reported to be associated with lumpy jaw (Keane et al., 1977; Burton, 1981; Samuel & Fowler, 1981; Antiabong et al., 2013a) were also described in our survey results. Our results support previous findings (Burton, 1981) that *F. necrophorum* is the leading causative agent, as our surveys found this species was reported in at least one culture by more than 40% of responding institutions. However, the frequency of occurrence in cultures was not reported to be high. Only 4.5% of respondents found this species in all cultures undertaken, whereas nearly 20% of institutions reported that they only detected *F. necrophorum* in cultures 'sometimes'. "Frequency of detection", as reported through the survey, was subjective; and the study would have benefited from a more precise and standardised scale for "frequency of detection", to determine more accurately the frequency of detection of all bacterial species cultured. As well, only half of our study's respondents used their zoo records when providing information about their bacterial culture, leaving open the possibility of imprecise reporting for this section of the survey.

Our results describe 20 bacterial species in association with lumpy jaw. The presence of specific bacteria will be dependent upon the existence of appropriate environmental conditions for the species. For example, the wetter climate in Europe will likely be favourable for the anaerobic bacteria *F. necrophorum* (Whittier & Umberger, 2009); and indeed *F. necrophorum* was the leading bacterial species reported in the European region (28.6%). The presence of this species in zoos and other captive environments is reported to be strongly influenced by environmental factors and management practices (Bennett et al., 2009); and it has the capacity to survive in the environment for up to two weeks (Whittier & Umberger, 2009). In European environments, levels of precipitation may also promote longer survival of bacteria in soils (Oliphant et al., 1984). Grass in the enclosure may act as a dietary source for macropods, and pathogenic bacteria could be transferred from the ground, to the oral cavity when macropods graze, as suggested by Bennett et al. (2009). Potentially the use of grass as a substrate may lead to a greater risk of exposure to, and ingestion of, pathogenic bacteria, as reflected by prevalence of lumpy jaw reported for the European region, where grass was the most commonly used substrate. It is also important to consider that controlling hygiene, and subsequent bacterial presence in grass enclosures, is challenging. It may be of benefit to use a substrate that is less likely to harbour bacteria, has the capacity to discourage grazing and the ingestion of bacteria from the ground, and also has the potential to enable effective enclosure cleaning procedures to be undertaken. The transfer of pathogenic bacteria from the environment to the animal, and vice versa, has also been hypothesised by Bennett et al. (2009). Bacteria associated with lumpy jaw are known to be transmitted via contact with contaminated material (e.g. bedding), through infected footwear and through vehicles used to transport infected animals (Whittier & Umberger, 2009). As zoo animals are routinely transferred between institutions (Hosey et al., 2013), there is a clear risk that bacterial transfer could occur; although as (Broom, 2003); Broom (2005) observes, increased stress from transfer between institutions may be a potential risk factor for lumpy jaw even in the absence of transmission of bacteria. Europe has a suitable climate for *F. necrophorum* to survive, and a high prevalence of lumpy jaw in countries across the region, as indicated by our survey results. Previous researchers have also reported *F. necrophorum*, along with many other bacterial species, in cases of lumpy jaw in the Australian region (Antiabong et al., 2013a). As several species of bacteria were only reported from the

European region, further investigations are required to determine the geographic distribution of bacterial species implicated in this disease.

3.5.5 Treatment

Diagnosis of lumpy jaw, and the delivery of preventive and/or remedial treatment for the disease, may be affected by the presence of veterinary personnel, physical and financial resources available at each zoological institution. Across institutions in our study, specific diagnostic methods undertaken for lumpy jaw reflected those recommended by Vogelnest and Portas (2008), and include radiography and microbial culture. Microbial culture is useful in the selection of appropriate antibiotics; however, in the majority of institutions, microbial culture was only used 'sometimes' (24%). With respect to treatment protocols, results from the survey indicate that institutions in our survey used the same treatment protocols as recommended by Butler and Burton (1980), Hartley and Sanderson (2003), Fagan et al. (2005), Vogelnest and Portas (2008) and Shah et al. (2016). Systemic antibiotics were the most commonly employed method of treatment (81.8% of institutions), with penicillins being the antibiotic of choice in the majority of institutions. Widely used surgical methods included tooth extraction and debridement of soft tissue, infected bone and tooth sulcus, and a notable number of institutions reported the regular use of AIPMMA beads. Although used successfully in several other species, including the treatment of dental abscesses in rabbits (*Oryctolagus cuniculus*) (Crossley & Aiken, 2004) and in the treatment of osteomyelitis in reptiles (Divers & Lawton, 1999), the success of AIPMMA beads has only been reported in macropods in a limited number of cases (Hartley & Sanderson, 2003; Grífols et al., 2013; McLelland, 2019). Beads have the added benefit of delivering antibiotic directly to the site of the lumpy jaw infection, therefore requiring less post-surgical intervention (Hartley & Sanderson, 2003). The benefit of having less post-surgical intervention could explain why this method was more commonly observed in the European region, where veterinary support was reportedly less frequent. Oral varnishes were used in 36.4% of institutions, at varying frequencies. Survey design did not permit us to determine whether respondents were reporting use of specific oral varnish products identified as beneficial for the treatment of lumpy jaw (Bakal-Weiss et al., 2010), or simply reporting use of the standard antibiotic gels often used in oral surgery. An assessment of the efficacy of oral varnishes, and other treatments for lumpy jaw, would benefit zoo veterinarians, and an improved survey design would correlate treatment type with eventual outcome. In addition, it would have been of use to obtain information from those institutions not affected by lumpy jaw, regarding their use of any preventive treatments, including vaccinations. This information may have provided important evidence about the efficacy of various preventive treatments, including vaccination, as a method of disease control.

Regardless of treatment, lumpy jaw is currently a leading cause of death in captive macropods, with euthanasia the likely outcome when prognosis is poor (Jackson, 2003; Vogelnest & Portas, 2008). This study's survey results indicate that lumpy jaw was responsible for the death of 82.8% of macropods diagnosed with the disease, with the majority of these being euthanased. Those housed in the Australian region were 3.4 times more likely to be euthanased than die without assistance (without euthanasia), compared to those housed in Europe. This greater risk of euthanasia in the Australian region may be a result of the perceived value or ease of replacement of macropods in the region. This could also be related in part to the consistent presence of a veterinarian in institutions in the Australian region; potentially providing a greater opportunity for early diagnosis and subsequent treatment. While our study did not find a significant relationship between daily veterinary presence and reports of lumpy jaw (p = 0.09), the relatively low p-value suggests that this potential risk factor may warrant further investigation.

3.5.6 Housing and husbandry

Macropod diet

Diet, and its delivery method, has previously been suggested to have a contributory role in the development of lumpy jaw (Burton, 1981; Gamble, 2004; Vogelnest & Portas, 2008). The present study was not able to determine an association between diet-related risk factors and the likely risk of developing lumpy jaw; however, a range of diets were reported among institutions. Pellets and hay were both used by a large majority of institutions (100% and 94.2% respectively), and notably, bread, which has been associated with the development of lumpy jaw (Hume et al., 1989; Jackson, 2003), was also part of the diet according to 19.2% of European feeding records. Across institutions, different categories of fresh foods were fed in differing proportions (42.3% and 87.8% for fruit and vegetables respectively). Fruit was found in nearly half of the European feeding records (48.1%), yet the feeding of diets that are high in fresh fruits has been associated with the occurrence of dental disease in other zoo species, for example the pygmy slow loris (*Nycticebus pygmaeus*) (Cabana et al., 2017). In addition, *F. necrophorum*, the bacterium named as a causative agent in lumpy jaw (Burton, 1981; Samuel, 1983; Antiabong et al., 2013a), has been reported to be able to survive on both fresh food and on pellets under certain environmental conditions (Butler & Burton, 1980). Therefore, in addition to the bacterium's survival in the wider environment, its persistence in dietary items may play a role in the risk of developing lumpy jaw. Environmental contamination of the diet has also been postulated as a risk factor for lumpy jaw, leading to suggestions that raising feeding stations off the substrate could prevent faecal contamination of food by pathogenic bacteria, such as *F. necrophorum*; thus reducing the risk of lumpy jaw (Burton, 1981). However, due to confounding across the feeding methods in the final dataset, our survey was unable to investigate if there was an increased risk of developing lumpy jaw in macropods fed using ground feeding methods (e.g. scatter feeding, feed containers on the ground) compared with those fed using off-ground feeding methods.

Macropod enclosures

Another potential risk factor related to housing and husbandry is the size of an enclosure in relation to the number of animals housed within it; particularly with respect to concentration of environmental faecal contamination, and potential links between higher densities of individuals and stress. Previous research (Ketz, 1997) has found increased incidence of lumpy jaw in zoos where macropods were kept in enclosures with < 10 m^2 of space per animal, with higher population density and subsequent faecal contamination, as well as potential chronic stress from overcrowding, postulated as potential factors. In the present study, the median enclosure size in the Australian region was almost twice that reported for the

European region (1137 m^2 and 750 m^2 respectively). Although enclosures were smaller across Europe, with the minimum reported enclosure space per animal being just 1.9 m^2, the median space per macropod reported by institutions exceeded the recommendations made by Ketz (1997); with enclosure sizes ranging from 17 m^2 to 100,00 m^2 per animal depending on institution. We note that we have no way of determining whether the enclosure sizes reported in the surveys were actual sizes or estimates.

This study also investigated enclosure environment as a potential risk factor for lumpy jaw; specifically, the relationship between substrate type and risk of ingestion of contaminated substrate. Ground feeding not only facilitates unintentional coprophagic behaviour, but also increases the ingestion of substrate. Substrate type may influence the presence of harmful bacteria, with bacteria preferring damp conditions that could be maintained by, for example, a grassy substrate (Ketz, 1997). In our study, grass was the most frequently reported substrate for macropods housed in institutions from the European region, and has the potential to harbour pathogenic bacteria associated with lumpy jaw, as previously discussed. However, it was not possible to calculate the relationship between substrate type and risk of developing lumpy jaw, due to ambiguity in the data relating to enclosures, substrate types, and the macropods that were housed within them. In the Australian region, mulch was the most frequently reported substrate; but again, no relationship could be calculated between substrate type and risk of developing lumpy jaw. Alongside the above-mentioned questions about grass, it could be argued that the composition of mulch (consisting of decaying leaves, bark, or compost), used more frequently in the Australian region, is also conducive to harbouring pathogenic bacteria, due to its ability to retain moisture to create a favourable environment for bacterial growth. However, it may be that this product has hidden benefits, as mulch is not part of a normal macropod diet and would be unlikely to be ingested by the animals. Therefore, the use of mulch as a substrate may reduce the risk of unintended ingestion of pathogenic bacteria from the environment when macropods forage in the enclosure.

Enclosure design and type, including mixed-species and walk-through exhibits, can have a wide range of positive and negative effects on health and behaviour (Kenny et al., 1993; Probst & Matschei, 2008; Marshall et al., 2016; Rendle et al., 2018). These aspects of housing are therefore important to consider as potential risk factors for disease. Mixed-species exhibits have become standard practice amongst zoos worldwide, with a range of taxa being successfully managed in this way (Dorman & Bourne, 2010), and this type of enclosure also provides enrichment for the inhabitants (Buchanan-Smith, 2012). However, species incompatibility in mixed-species enclosures may act as a potential stressor (Rendle et al., 2018). Another type of housing system, the walk-through enclosure, provides a means for zoos to actively encourage interaction between humans and animals (Morgan & Tromborg, 2007; Hosey et al., 2013), and walk-through enclosures are frequently used for housing macropods in zoos (Sherwen et al., 2015). However, both mixed-species and walk-through enclosures have the potential to adversely affect the occupants, due to the potential for increased risk of stress, through the presence of unsuitable cage-mates or humans, and the risk of disease transmission between different species (Coe, 2003; Probst & Matschei, 2008; Rendle et al., 2018). Conversely, single-species non-walk-through housing systems may reduce the potential of both species incompatibility and human-induced stress (e.g. close proximity), and the occurrence of disease transmission between species. Our results for the Australian region support this hypothesis; macropods in this region were reported to be most commonly housed in single-species systems, and as previously stated, macropods housed in the Australian region had a significantly lower prevalence of lumpy jaw than those housed in the European region. However, single-species enclosures were also reported as the most commonly used housing system in Europe, where the prevalence of lumpy jaw was significantly higher than the Australian region, subsequently challenging the aforementioned hypothesis. These results could be confounded by other aspects of the macropod housing and husbandry in Europe, including substrate, stocking density and species housed.

Other confounding factors could include a species-specific susceptibility lumpy jaw, as the macropod species more commonly observed in both walk-through and mixed-species enclosures include those with the greatest reported prevalence of lumpy jaw, according to the findings of this study and also those reported by Sherwen et al. (2015). Equally, the number and proximity of visitors in walk-through enclosures can influence stress levels (Morgan & Tromborg, 2007). However, even after undertaking additional physiological measures for stress hormones, Sherwen et al. (2015) were unable to conclusively determine that visitor presence negatively impacted macropods. Species susceptibility may have played a role in these results, as the western grey kangaroo, a species observed in the Sherwen et al. (2015) study, is considered to be a particularly resilient macropod species around humans (M. Lynch, personal communication, 12th November 2016). The potential effects of visitors acting as a potential stressor have not been well studied or clarified; and as Sherwen et al. (2015) have demonstrated, are challenging to measure.

Staff and equipment biosecurity

Enclosure hygiene has also been reported as a potential risk factor for lumpy jaw; with recommendations for the regular removal of faecal contamination from enclosures (Burton, 1981). Visitors in walk-through enclosures, and staff entering enclosures, may likewise pose a threat with respect to introducing bacterial contamination to the environment, especially in the absence of adequate biosecurity measures. In the present study, institutions in which keepers did not undertake biosecurity with respect to footwear were 4.8 times more likely to observe cases of lumpy jaw (OR 4.8, p = 0.01). These findings suggest that effective control of environmental contamination through adequate keeper biosecurity protocols could be a key factor in the control of pathogenic bacteria and subsequently, the control of lumpy jaw disease in captive facilities. In addition, institutions that did not undertake tool cleaning after each use, or between enclosures, were significantly more likely to report an incidence of lumpy jaw (p = 0.02 and p = 0.04 respectively). Appropriate biosecurity measures have been shown to deliver measurable benefits when managing other diseases in similar contexts (for example footrot), and given the importance of identifying housing and husbandry factors related to management of lumpy jaw, this aspect of our findings would benefit from additional research.

Enclosure hygiene is not limited to the enclosure's substrate; it also includes its contents, specifically vessels used for feed and water. Bacteria can be disseminated into the environment, during active cases of lumpy jaw, in the animal's saliva (Broadley & Schweon, 2017). During observations undertaken in this study of macropods drinking, saliva was noticed to enter the water, potentially leading to bacterial contamination of the water vessel from infected animals. As discussed earlier, some bacterial species have the ability to survive in damp conditions, and *F. necrophorum*, a pathogen named in association with cases of lumpy jaw, can survive in water (Broadley & Schweon, 2017). Water vessels may therefore be an important potential source of contamination in those institutions where daily washing is not carried out. In this study, European institutions were 7.6 times more likely to have lumpy jaw when daily cleaning of water containers was not conducted, although this result was not statistically significant ($p = 0.07$). However, a greater sample size would provide results that are more conclusive as to the likelihood of water vessels being a potential source of contamination. It should be noted that footwear and tool hygiene may be a proxy for general biosecurity standards used in zoos, and may be associated with fomite transmission of pathogens (Firestone et al., 2011). Future studies should focus on this risk factor.

3.5.7 Limitations of this study

Written surveys are an excellent tool for the collection of data from multiple institutions without the economic and logistical difficulties of direct visitation and associated travel; however, they also have many potential drawbacks. High response rates are hard to achieve, and using surveys, or methods of data collection that suits the target audience, is recommended as a way of addressing the issue of low response rates to researchers' questions (Plowman et al., 2013). To make use of the advantages of various survey methods, and to reduce non-response bias, the present study used a mixed-methods approach (online and paper-based surveys), as recommended by Schaefer and Dillman (1998). To achieve statistical strength with 95% confidence, a sample size of 139 respondents was required (Sergeant, 2018). Initially, we received responses from 122 institutional respondents, however, after selecting only those which had fully completed the survey, our remaining institution population stood at

80. Given our sample size was lower than the minimum size required for 95% confidence (Sergeant, 2018), our results may have an increased risk of not truly reflecting the prevalence of lumpy jaw in captive macropods, or the risk factors associated with the disease.

Surveys are subject to both selection and response bias (Rindfuss et al., 2015; Christley, 2016), and the subsequent response rates are often affected by a bias developed from the selection of survey participants (Rindfuss et al., 2015). In our study, participants were sourced mainly through their membership to zoological associations; which requires paid subscription. Potentially, this may have biased our participant selection process towards those zoos that were financially stable and therefore able to pay subscriptions. Furthermore, some zoological associations encourage their member institutions to partake in research so strongly that research participation is an essential part of membership (Plowman et al., 2013). We sought to reduce selection bias by undertaking extensive internet searches for institutions independent of zoological associations; however, we did not request information about whether our respondents held membership/s with any zoological associations, and we expected it was likely that most respondents were members of one of the many available associations. Potentially, however, respondents from smaller independent zoos may have had fewer available staff and resources to devote to research requests; and these may have been the institutions less likely to have responded to our survey; thus skewing our results towards the larger, more resource-rich institutions. In addition, a relatively large proportion of surveys were completed by veterinarians (37.5% of responding institutions), which may have influenced the results. Zoo veterinarians may not have had first-hand knowledge to answer many of the management and husbandry questions, leading to the risk that answers to those questions may reflect individual opinion as to what should be done, rather than a reflection of what was actually carried out.

Poor survey design and delivery can lead to non-response (Mellen, 1994) and the survey would have benefited from a simpler structure, and from being shorter in length. Additionally, the university portal used to support the survey had several technical issues; hampering smooth delivery of the survey. Another problem with the survey was the inability for respondents to save their online answers, and complete the survey over multiple sessions. This too may have affected the response rate.

Surveys have the advantage of collecting data from a broad geographic area with minimal effort (Thrusfield & Christley, 2018). However, our survey was delivered across geographic regions where several languages are spoken; potentially resulting in misinterpretations of the survey questions by some respondents. Some of the answers and information given in completed surveys indicated that respondents were confused about some of the terms in the questions such as sex and age ratios; it was clear that we lost a number of data points for this area of the survey.

Survey reliability is one of the biggest disadvantages of collecting epidemiologic data, (Glatt et al., 2008; Van Gelder et al., 2010; Thrusfield & Christley, 2018). Reliance is placed upon the respondent to provide true and accurate data, however with sensitive topics, such as enclosure hygiene, some institutions may be inclined to provide data on what should occur, rather than what actually does occur. The large number of veterinarians completing the survey may have influenced the results, given that routine husbandry is not an activity usually carried out by a veterinarian. Furthermore, several institutions reported that they acquired their data from zoo staff rather than from records, which could lead to inaccuracy due to human (memory) error. However, records themselves are not always accurate, relying on the person who completed them for accuracy. Respondent bias is often observed in surveys, including a bias towards survey completion by participants who perceive benefits from such completion (Thrusfield & Christley, 2018). In our study, this may have manifested as a bias by institutions to complete the survey if they had experienced lumpy jaw recently; we note that the majority of responding institutions (71.3%) had reported lumpy jaw in the last five years.

Surveys are limited in their capability to collect detailed information (Thrusfield & Christley, 2018), and in the present study, missed opportunities to collect important data were identified. For example, questions relating to the use of vaccinations could have been explored further, by requesting exact details of the vaccine(s) and the vaccination program used. This should be a priority question for future research.

3.5.8 Recommendations

As results from this survey have shown, lumpy jaw is a complex and often fatal syndrome with multiple aetiologies. Although if detected and treated in the early stages, prognosis is improved (Blyde, 1999). A team approach to the detection of signs of lumpy jaw should be routine practice in zoos that house macropods. Good communication between keepers and veterinarians is essential, especially when veterinary care is not constantly available at the zoo site. With appreciation for the financial implications and possible confounders, our study's findings indicate that zoos may benefit from the daily presence of a veterinarian with expertise in macropod health and disease. Regular health assessments involving general anaesthetic, to facilitate a detailed examination of the oral cavity, should also be implemented as part of standard macropod management.

Poor hygiene in enclosures increases the risk of exposure to pathogenic bacteria (Rees, 2011; Hosey et al., 2013), especially those associated with lumpy jaw (Burton, 1981), however, our study's findings indicate that poor hygiene within macropod enclosures may be specifically associated with a lack of biosecurity measures carried out by staff entering enclosures. Results from this study suggest that biosecurity is an important aspect associated with the development of lumpy jaw, with significant associations found between cleaning of footwear ($p = 0.01$), cleaning tools after each use ($p = 0.005$) and cleaning tools between enclosures ($p = 0.02$), and the development of lumpy jaw. The introduction of zoo policies for the use of footbaths, with an antibacterial disinfectant, to be used by all staff, and potentially visitors, entering and leaving walk-through enclosures, may reduce the risk of bacterial contamination of enclosures. In addition, zoo personnel should ensure that all tools and equipment are disinfected after each use, and between enclosures. From this research, it is also likely important that water vessels are cleaned on daily basis. However, as with any survey-based studies, it is challenging to make conclusive recommendations without further investigations that control for confounding.

3.5.9 Future research

Further research will be invaluable to investigate the potential impacts of human traffic and biosecurity on zoo-housed macropod health and welfare. Recent research into the impact of walk-through enclosures on the welfare of macropods was largely inconclusive (Sherwen et al., 2015). However, the Sherwen et al. (2015) findings may have been limited by the relatively small sample size (n = 15), and differences in enclosure occupants, namely the species and population dynamics of those housed and the subsequent effect this may have had on the behaviours being measured. Likely human influence on the development of lumpy jaw was identified in this current study, with an association found between biosecurity and occurrence of the disease. However, we were not able to examine whether enclosure type, especially enclosures that could be accessed by visitors, was correlated with the development of lumpy jaw; therefore, further research in this area is necessary. We recommend further epidemiological studies be undertaken to investigate the association between enclosure type, environmental contamination of walk-through enclosures, and the development of disease. Targeted interventions could be used to determine whether the incidence of lumpy jaw decreases as a result of implementation of biosecurity changes for zoo personnel and visitors in walk-through enclosures. We note that multiple concurrent management changes can be challenging to interpret. We also note that by nature, zoos are dynamic, with frequent changes to management and husbandry to meet institutional needs and development in knowledge. However, such changes can confound and impact research findings. Ideally, a multi-institutional study that aims to control for some of these confounding variables would provide the most insight into the potential risk factors identified in this survey. Specific types of observational studies, such as prospective cohort studies, would be beneficial for detecting if chronic exposure to visitor presence and/or environmental contamination are correlated with the development of diseases such as lumpy jaw. Equally, research into the efficacy of vaccinations used in footrot, and often used in macropods to prevent lumpy jaw (Jackson, 2003), should be carried out. Case control studies aimed at evaluating prophylactic treatments against disease risks would also be beneficial, such as investigating the relationship between use of vaccinations and likelihood of disease.

3.6 Conclusion

Lumpy jaw is a disease affecting captive macropods that causes high morbidity and mortality, raising welfare concerns for captive macropods worldwide. The results from this research provide insight into the current prevalence of lumpy jaw in captive macropods housed across two regions (Australia and Europe), and show that the aetiology of lumpy jaw remains complex and multifactorial. The prevalence of lumpy jaw found in this study is higher than previously reported, and results suggest that geographical region and climate may play a role in the development of disease. To reduce the incidence of lumpy jaw, husbandry practices to control pathogenic bacteria should be improved, and investigations into links between the disease and biosecurity (for both personnel and enclosures) should be undertaken.

Multi-zoo studies are complex, especially when utilising tools such as surveys, which have known limitations and challenges to implementation. Nevertheless, this type of research is essential for detecting the extent of diseases such as lumpy jaw in zoos, and this study has shown that surveys can be a useful tool in the collection of data from a broad geographical area and across multiple institutions. As we seek to achieve the gold standard of care for animals in captivity, this survey has managed to extract important new information about this refractory disease. The results from this study will provide a foundation for further investigations; and will help to define what is essential for the reduction or elimination of diseases such as lumpy jaw in captive contexts. As this information emerges, institutions will be better able to provide appropriate living conditions for macropods, in order to establish and maintain good health and welfare for captive macropods over the long term.

Chapter 4

A retrospective cohort study of lumpy jaw in captive macropods across two regions: Australia and Europe

4.1 Introduction

Lumpy jaw is a disease that has been reported in macropods in zoological collections worldwide (Butler & Burton, 1980; Vogelnest & Portas, 2008; Kido et al., 2013; Sotohira et al., 2017a); however, epidemiological data examining the level of risk and factors associated with the development of clinical disease are limited. The frequent occurrence of lumpy jaw in zoo macropods compared to their wild counterparts suggests an association between the disease and aspects of the captive environment (Burton, 1981; Jackson, 2003; Bodley et al., 2005; Vogelnest & Portas, 2008; Sotohira et al., 2017a). Therefore, an epidemiological study identifying the factors associated with the occurrence of lumpy jaw would aid in the development of preventive strategies, ultimately reducing morbidity and mortality rates of this refractory disease.

Lumpy jaw is a disease of multifactorial aetiology, and potential triggers for the disease have been proposed to originate from both environmental and animal-centred sources. These risk factors include feeding strategies, high stocking densities, enclosure contamination with pathogenic agents in the faeces, and stress (Calaby & Poole, 1971; Finnie, 1976; Butler & Burton, 1980; Burton, 1981; Ketz, 1997; Brookins et al., 2008; Vogelnest & Portas, 2008). In captivity, the location of the host institution and institutional management strategies predominantly control these factors, including: the diet and methods of dietary presentation, the number of macropods housed in an enclosure and subsequent levels of hygiene, and potential sources of stress that may impact immunity, such as visitor presence and proximity, confinement, transport and environmental conditions (Broom, 2005; Morgan & Tromborg, 2007). Precursors for lumpy jaw may also involve those that originate from the host, with the presence of periodontal disease and the processes of tooth eruption and molar progression cited as primary factors leading to the disease (Clarke, 2003; Jackson, 2003; Vogelnest & Portas, 2008). The rates at which macropod teeth erupt, and at which molar progression occurs, vary between genera and species, and correlate with age (Kirkpatrick, 1964; Newsome et al., 1977; Clarke, 2003; Kido et al., 2018); therefore the association between host-related dental development and the incidence of lumpy jaw potentially varies between macropod genera and age. In some macropod species, sexual dimorphism in dentition and body mass is reported (Newsome et al., 1977; Mysterud, 2000); however, a sex bias in the development of lumpy jaw is yet to be identified. While these host-related risks for lumpy jaw may be challenging to control; exposure to environmental triggers for the disease are predominantly manageable by the institution. However, there are institutional differences in management practices, species housed, and geographic location; and the effects of these differences on the incidence of lumpy jaw have not been substantially investigated.

Lumpy jaw in captive macropods has been reported across multiple genera and countries (Chapter 1, pp. 25 – 27), with variability in the prevalence of the disease between genera, within a genus, and even within species (Vogelnest & Portas, 2008), along with differences of prevalence between reporting institutions (Vogelnest & Portas, 2008; Kido et al., 2013). Studies of lumpy jaw involving *Macropus* spp. form the majority of publications, including red-necked wallabies (Kane et al., 2017), red kangaroos (Brookins et al., 2008) and tammar wallabies (Butler, 1981), with reported prevalence ranging from 0% in the tammar wallaby (Vogelnest & Portas, 2008) to 58.3% in the red kangaroo (Butler & Burton, 1980). A retrospective study of lumpy jaw, using clinical records, which followed the health of macropods from birth to death in Japan, described a considerably higher prevalence of the disease at 40% (Kido et al., 2013), than that found for the same species in a necropsy study at an Australian institution at 9.5% (Vogelnest & Portas, 2008). This supports the climate-associated risk factor proposed by others where colder temperatures drive disease presentation through immunosuppression (Burton, 1981; Oliphant et al., 1984). Yet despite potential climatic differences, a similarly high prevalence of 40% in *Macropus* sp. was found in an Israeli institution (Bakal-Weiss et al., 2010), although this prevalence may be associated with genera-specific differences in anatomical characteristics (grazer/browser/mixed feeder-type), or behavioural characteristics, such as flightiness (Jackson, 2003). In addition to geographic and genera-specific influences on the occurrence of lumpy jaw, study design and institutional differences in management could be responsible for the variation in reported prevalence. One multi-institution and 'transatlantic' investigation of lumpy jaw described a prevalence of lumpy jaw of between 10% and 20% (Ketz, 1997), although this was collectively across regions and institutions, and therefore does not specify which countries, regions and genera were affected. The variability in the reported prevalence of lumpy jaw within and across institutions, regions and species, is likely affected by significant confounding which complicates the epidemiological analysis of risk factors for this disease.

Lumpy jaw studies to date have been limited by the composition of the study population, the application of crude disease frequency measures (prevalence across species and prolonged time frames), and the inherent complications of confounding given the wide variety of environmental and host influences on this disease which undermine the ability to derive comparable study populations in captivity. The multifactorial and chronic nature of the disease further complicates research design, as experimental infection studies cannot be applied, and prospective cohort studies ideally require fixed management of study populations over extended time frames, conditions that are not easily achieved within the captive environment. The majority of reports have used biased study populations, where prevalence is reported either from deceased individuals (e.g. Vogelnest & Portas, 2008), or by comparing deaths from lumpy jaw to all other causes (Butler, 1981). These studies do not include data from macropods that were successfully treated, and survived a case of lumpy jaw, nor the entire population at risk; therefore, these results do not reflect the full extent of the disease in 'live' captive populations. Importantly, most studies describe period prevalence (cases of lumpy jaw over time periods that may extend to years), rather than an incidence rate that takes into account the animal time at risk and provides for dynamic populations where animals enter and leave the study population throughout the period of reporting (Thrusfield & Christley, 2018). Whilst prevalence reflects the disease burden in a given population over time, the incidence rate (animals developing disease/total animal time at risk) and incidence rate ratio (comparison between incidence rates for different risk factors) are better suited to investigating disease aetiology and risk factors (Noordzij et al., 2010), and are the more appropriate measurements to apply to captive study populations of macropods with lumpy jaw.

This is particularly the case given that lumpy jaw prevalence will likely be inflated by the chronicity of disease if datasets cover extended time periods.

4.2 Aims

The reported frequency of lumpy jaw in captive macropod populations worldwide both compels and provides for epidemiological investigations of the disease, despite the inherent biases and issues of confounding for these study populations. Using zoo-based records, the aims of this research were to determine the regional prevalence of lumpy jaw in captive macropods housed across two regions where macropods are popular exhibits: Australia and Europe. In addition, we aimed to systematically examine temporal trends in incidence of the disease (the incidence rate [IR]), and explore risk factors for the development of lumpy jaw (the incidence rate ratio [IRR]).

4.3 Methods

The selection of institutions involved in this study, including the methods used for the extraction of data, institutional selection criteria, and the retrospective period used for this study, are discussed in the General Methods section, Chapter 2. For privacy, institutions are anonymised: institutions in the Australian region are identified using the prefix A, with the four individual institutions referred to as Zoos A1 - A4 respectively, and European institutions have the prefix E, and are referred to as Zoos E1 - E4. Additional methods that are specific to this chapter are discussed below.

4.3.1 Selection of study population

All macropods recorded as being housed at participating institutions during the study period were included in the analyses. However, the literature suggests that lumpy jaw is not detected in pouch young (PY) (Kido et al., 2013), and arguably, PY would not be exposed to all of the same risk factors for lumpy jaw as adults; therefore, we only included macropods that were > 0.2 years (73 days), to remove the influence of outliers.

4.3.2 Inter-zoo transfers

Inter-zoo transfers were recorded on the Specimen Reports as a transfer between 'physical holders'. Any transfer between physical holders was counted as one inter-zoo transfer up to the point of entry into its current location. Macropods that entered a zoo on multiple occasions had their total number of transfers calculated. Animals that were brought in from the wild, and remained in captivity (given a GAN), were reported as one transfer, unless further information about them was known. A reported macropod escape, and subsequent return to an enclosure, was not classed as a transfer. All animals with an unrecorded history were reported as 'unknown'.

4.3.3 Intra-zoo transfers

A combination of institutions' Specimen Reports, Notes and Observations, and Enclosure Reports were used to identify the number of intra-zoo transfers each animal experienced. All transfers between 'on exhibit', 'off exhibit' and 'hospital' transfers were counted. Records also indicated that transfers occurred between hospital enclosures, and these were also included in the number of intra-zoo transfers. Not all institutions recorded the intra-zoo transfers of their animals, in which case these individuals were recorded as 'unknown'. Intra-zoo transfers recorded on ZIMS were for the animal's lifespan at the zoo, therefore the figures provided for total intra-zoo transfers reflect the number of enclosure moves that each animal experienced for the total lifespan at the institution, rather than solely for the study period.

4.3.4 Statistical analyses

Prevalence and odds ratio calculations

Data were entered into Microsoft® Excel 2016 for initial exploration. Prevalence was defined as the proportion of macropods within the population identified in the records as being affected by lumpy jaw at some point during the retrospective period of 1st January 1996 to 31st December 2015. Prevalence and confidence intervals were calculated using the exact binomial method (Ross, 2003) and the calculation of odds ratios were performed using Epitools (Sergeant, 2018). Chi-square tests for measures of significance were used when all categories were greater than five, and two-tailed

Fisher's exact tests when any one category was less than five. Measures of difference for geographical region were assessed using the Chi-square test as calculated in Epitools (Sergeant, 2018) with the measure of significance taken at $p \leq 0.05$.

Incidence rate calculations

Monitoring time was defined as from the initial date of arrival into an institution (for example, from birth or transfer in) or data was trimmed from 1st January 1995 (whichever was the later), until date of first incidence of lumpy jaw, death, or lost-to-follow-up (whichever occurred first). The follow-up date for some individuals went into 2016 (until 28th November 2016, the last date when data was extracted from ZIMS). Animals with no date of arrival or departure were removed from IR and IRR analyses. Incidence rate ratios were calculated for animals grouped by calendar period (1995 – 1999, 2000 – 2004, 2005 – 2009, 2010 – 2016), age group (< 1 year, 1 – 4 years, 5 – 9 years, ≥ 10 years), sex (male, female), genus, and institution. Incidence rates were calculated by dividing the number of cases by the monitoring time at risk, and presented per 100 animal years. Ninety-five percent confidence intervals were derived using the exact Poisson method.

Analyses involving between-group comparisons were conducted using Poisson regression modelling, with an offset term set to the number of monitoring years. Adjusted IRR were calculated by setting a reference category for each factor and comparing each of the remaining levels to the corresponding reference category, i.e. the reference category was '1995 – 1999' for calendar period; '< 1 year' for age group, and 'females' for sex. Comparisons between institutions were conducted using the institution with the lowest unadjusted incidence rate for each region separately. All models included calendar period, age group, sex, and zoo as separate terms to facilitate estimation of an adjusted IRR. The IR and IRR presented were calculated in R version 3.3.3 (R Core Team, 2013).

4.4 Results

4.4.1 Summary

Examination of 6178 animal records revealed that 2759 macropods were housed across the eight institutions between 1st January 1995 and 28th November 2016. The population considered at risk (> 0.2 years) was comprised of 2054 macropods; 1620 in Australia and 434 in Europe. Table 4.1 presents a summary of the study population.

Table 4.1: Summary of macropods housed at eight zoological institutions across Australia and Europe between 1st January 1995 and 28th November 2016. LJ = lumpy jaw. For sex, 'm' = male, 'f' = female, and 'ud' = undetermined.

Region	Institution	Total population	Sex (m.f.ud)	Genera	Species	Total LJ cases
Australia	A1	643	257.359.27	5	11	89
	A2	354	151.190.13	7	17	66
	A3	305	100.194.11	7	15	22
	A4	318	135.165.18	8	19	47
Europe	E1	60	25.34.1	1	1	10
	E2	224	109.112.3	3	8	25
	E3	84	41.35.8	2	5	11
	E4	66	32.28.6	1	1	7

4.4.2 Prevalence

The prevalence of lumpy jaw in captive macropods housed in the Australian region between 1st January 1995 and 28th November 2016 was 13.8% (95% CI: 12.2 - 15.6, n = 224/1620); slightly greater than that calculated for the European region at 12.2% (95% CI: 9.3 - 15.7, n = 53/434), although the difference in these results was not statistically significant (p = 0.45).

4.4.3 Incidence rates (IR) and incidence rate ratio (IRR)

Region

The IR for lumpy jaw in the Australian region between 1st January 1995 and 28th November 2016 was 5.7 cases/100 animal years (95% CI: 5.0 - 6.6, n = 206/1513), which did not significantly differ from the European region at 4.9 cases/100 animal years (95% CI: 3.6 - 6.5, n = 50/389).

Sex

Macropod sex was a significant risk factor for developing lumpy jaw; although the relationship was only established at the regional level (Table 4.2). Males in European institutions were two times more at risk of developing the disease than females (IRR 2.02, 95% CI: 1.08 – 3.83, p = 0.03) (Table 4.3), whereas macropod sex was not found to be a significant factor for the Australian region (IRR 1.08 cases/100 animal years, 95% CI: 0.80 – 1.44, p = 0.611).

Table 4.2: Incidence rates (cases/100 animal years) and 95% confidence intervals by sex for lumpy jaw in macropods in four Australian and four European institutions between 1st January 1995 and 28th November 2016.

Region	Sex	Cases	Years at risk	Incidence rate	95% CI
Australia	f	138	2330.8	5.9	5.0 - 7.0
	m	68	1222.2	5.6	4.3 - 7.1
Europe	f	25	604.2	4.1	2.7 - 6.1
	m	25	401.8	6.2	4.0 - 9.2

Age

The age at which lumpy jaw was first detected for macropods within the study ranged between 0.3 years and 18.9 years. The mean (SD) age of onset in this study population was similar in both regions (Australia 5.6 years [±3.6]; Europe 6.0 years [±5.1]), although variation was noted between species (results relating to age of onset are presented in Chapter 5, Table 5.12, p. 174). The likelihood of developing lumpy jaw increased with age (Figure 4.1), with macropods aged over 10+ years significantly more at risk of developing disease for both Australia (IRR 7.63, 95% CI: 4.06 – 15.20, p = < 0.001), and Europe (IRR 7.38, 95% CI: 2.50 – 24.85, p = < 0.001) (Table 4.3).

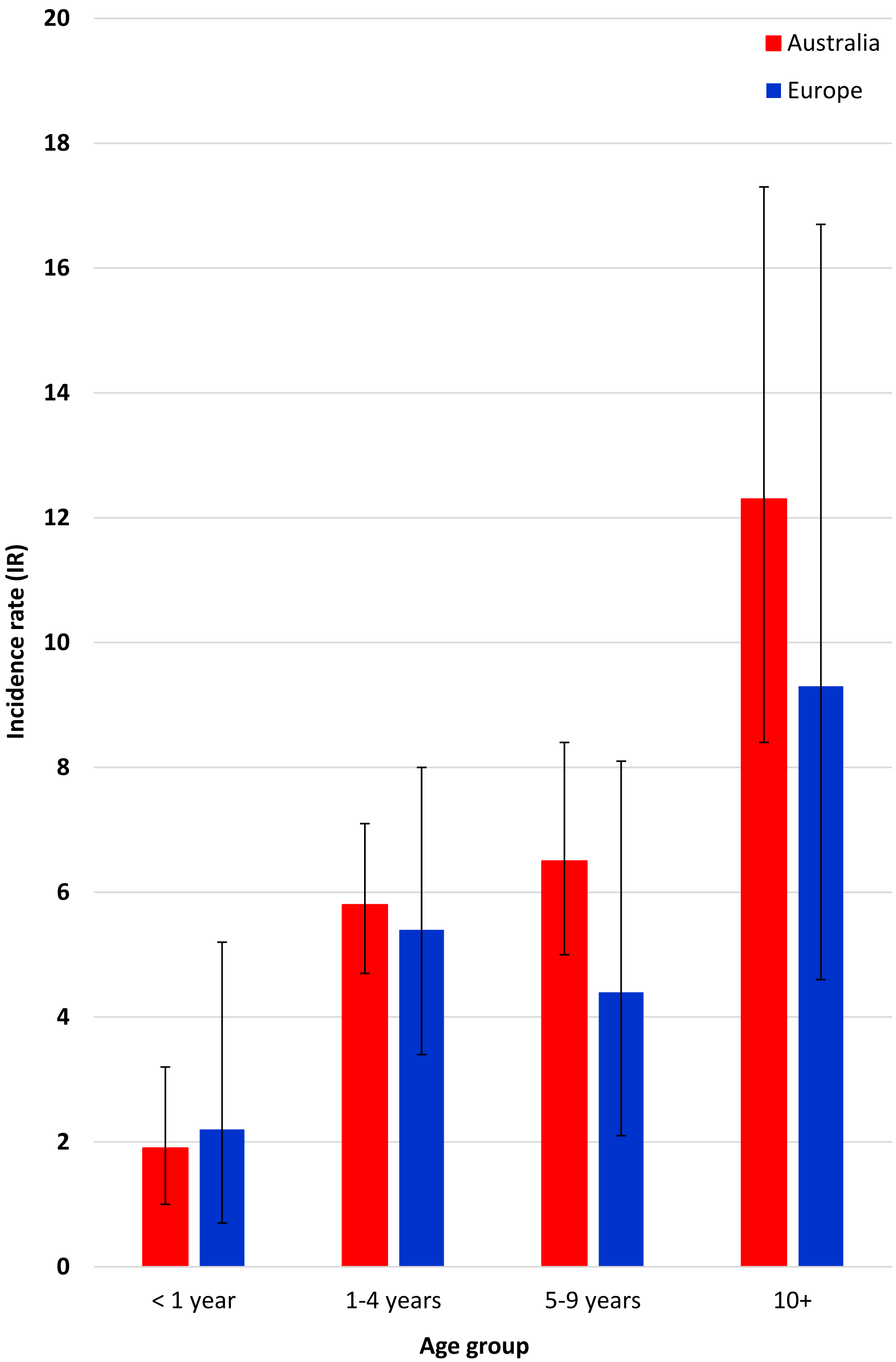

Figure 4.1: Incidence rate (cases/100 animal years) and 95% CI for lumpy jaw by age group, for macropods housed at eight zoological institutions across Australia and Europe between 1st January 1995 and 28th November 2016.

Genus

Lumpy jaw was identified in all genera within the Macropodidae family, including *Dorcopsis*, although in this genus disease was noted outside of the study period. The combined regional data determined the greatest IR was in *Wallabia* (IR 7.1 cases/100 animal years, 95% CI: 3.2 – 13.5), closely followed by *Petrogale* (IR 6.8 cases/100 animal years, 95% CI: 5.3 – 8.7), and *Macropus* (IR 6.5 cases/100 animal years, 95% CI: 5.5 – 7.6), although the difference between these genera was not significant. However, the IRs in *Wallabia*, *Petrogale* and *Macropus* were all significantly greater than the IR reported for *Setonix* (IR 1.0 cases/100 animal years, 95% CI: 0.4 – 2.1) (Figure 4.2).

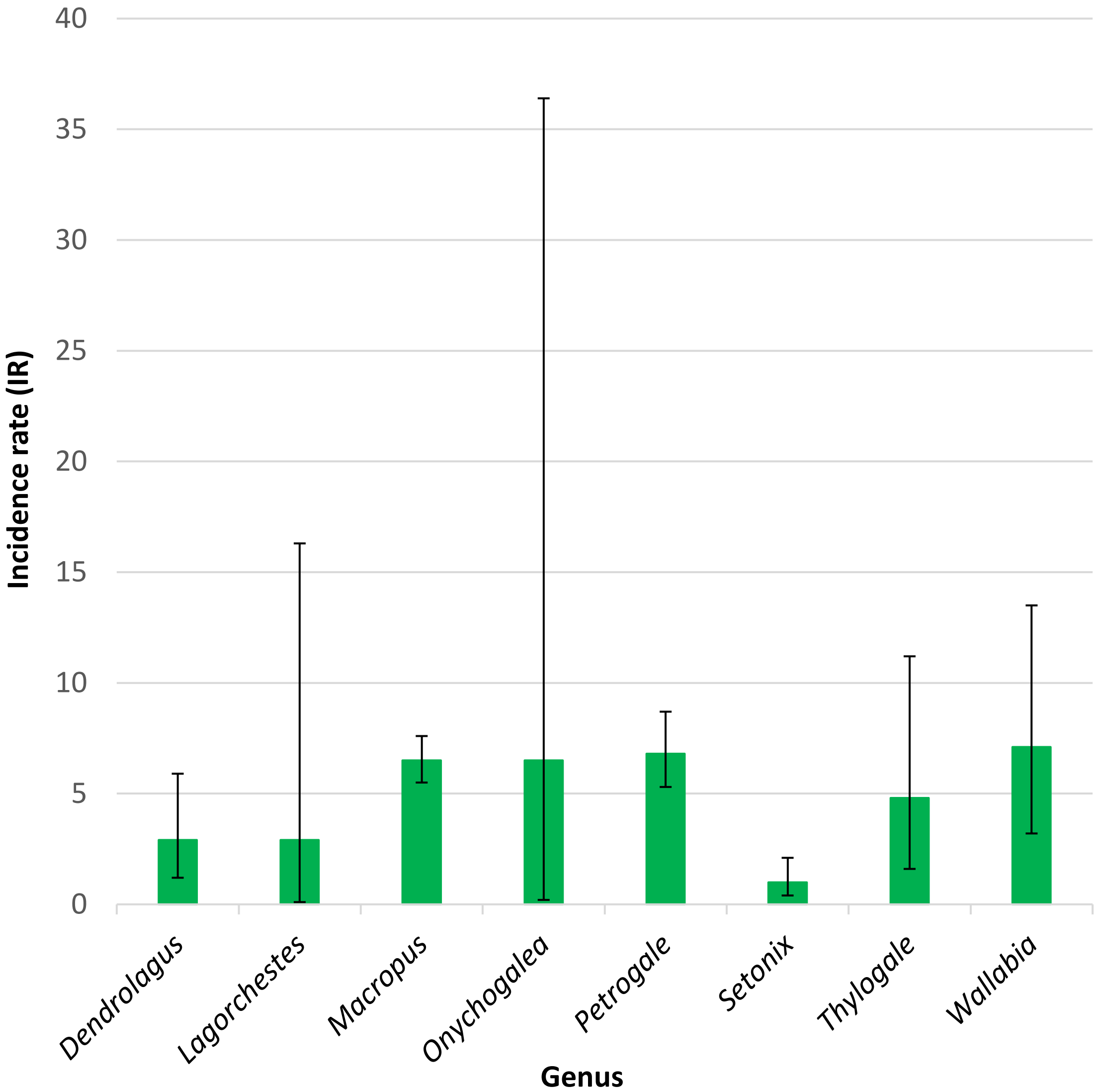

Figure 4.2: Incidence rates (cases/100 animal years) and 95% CI for lumpy jaw by genus for macropods housed at eight zoological institutions across Australia and Europe between 1st January 1995 and 28th November 2016.

Institutional IR and IRR - Australian region

Institutional IRs indicated that Zoo A3 had the lowest incidence of lumpy jaw of all Australian institutions throughout the study period, with only 16 cases of lumpy jaw reported between 1995 and 2016 (IR 2.4 cases/100 animal years, 95% CI: 1.4 - 3.9) (Figure 4.3). The modified Poisson regression model estimated the risk of developing lumpy jaw to be significantly greater for macropods housed at all Australian institutions, when compared to Zoo A3 (Zoo A1: $p < 0.001$; Zoo A2: $p < 0.001$; Zoo A3: $p = 0.013$) (Table 4.3).

Institutional IR and IRR - European region

In the European region, the lowest IR was observed at Zoo E4 (IR 3.6 cases/100 animal years, 95% CI: 1.4 - 7.3) (Figure 4.3); however the risk of developing lumpy jaw was not statistically different between any of the European institutions ($p > 0.05$) (Table 4.3).

Study period - Australian region

The incidence of lumpy jaw remained relatively stable in the Australian region throughout the study period, although a slight reduction in incidence was observed in recent years (2010 – 2016). However, the risk of developing lumpy jaw was not significantly different between any two of the specific 5-year periods examined ($p > 0.05$) (Figure 4.4; Table 4.3).

Study period - European region

The last 10 years (2005 – 2016) have seen a significant increase in the incidence of lumpy jaw in the European region (Figure 4.4). In recent years (2010 – 2016), macropods were nearly seven times more at risk of developing lumpy jaw than when recording began in 1995 (IRR 6.94, 95% CI: 1.96 – 44.18; $p = 0.010$), and were at greatest risk during 2005 – 2009 (IRR 7.39, 95% CI: 2.06 – 47.21, $p = 0.008$) (Table 4.3).

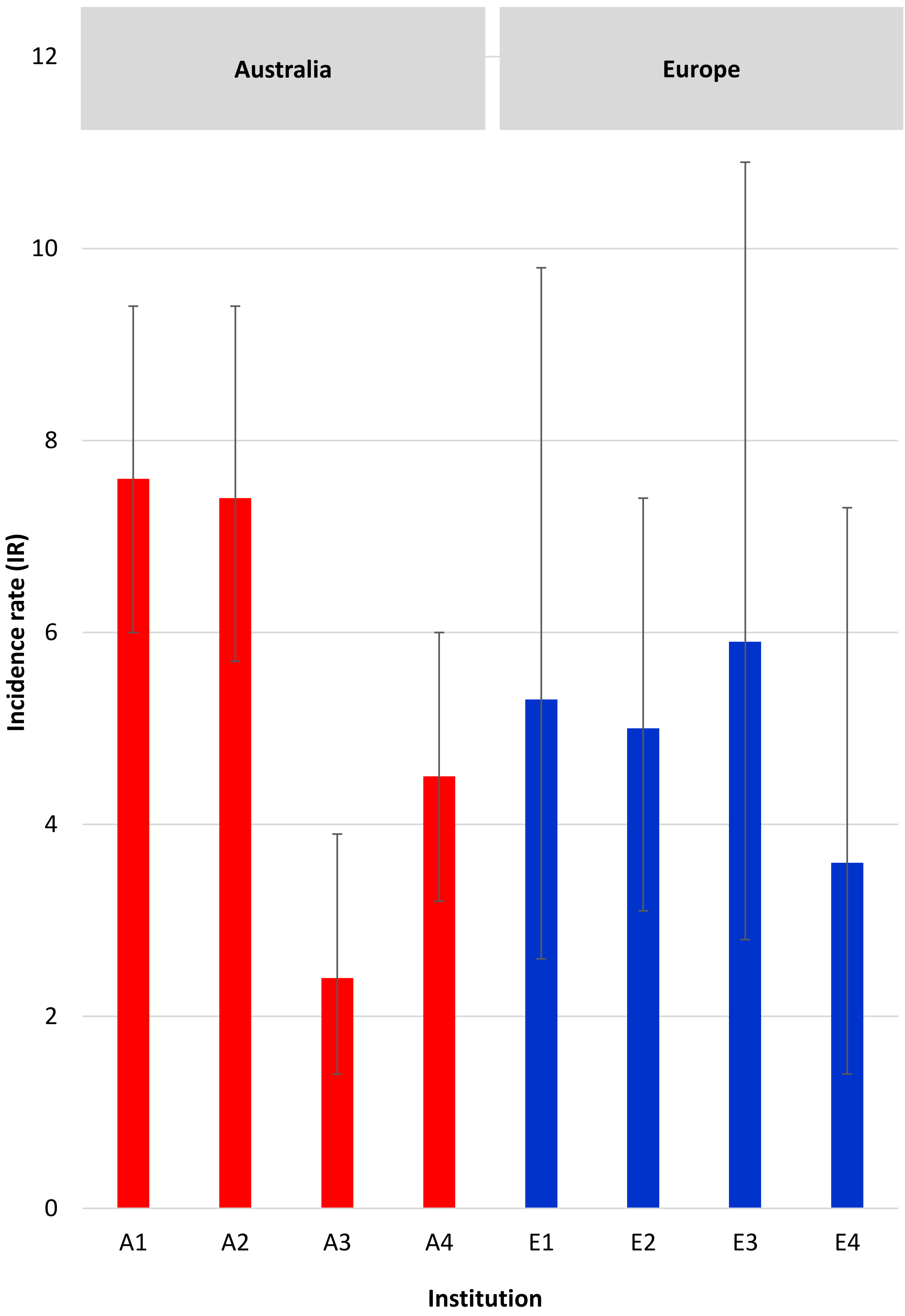

Figure 4.3: Incidence rates (cases/100 animal years) and 95% CI for lumpy jaw by institution for macropods housed at eight zoological institutions across Australia and Europe between 1st January 1995 and 28th November 2016.

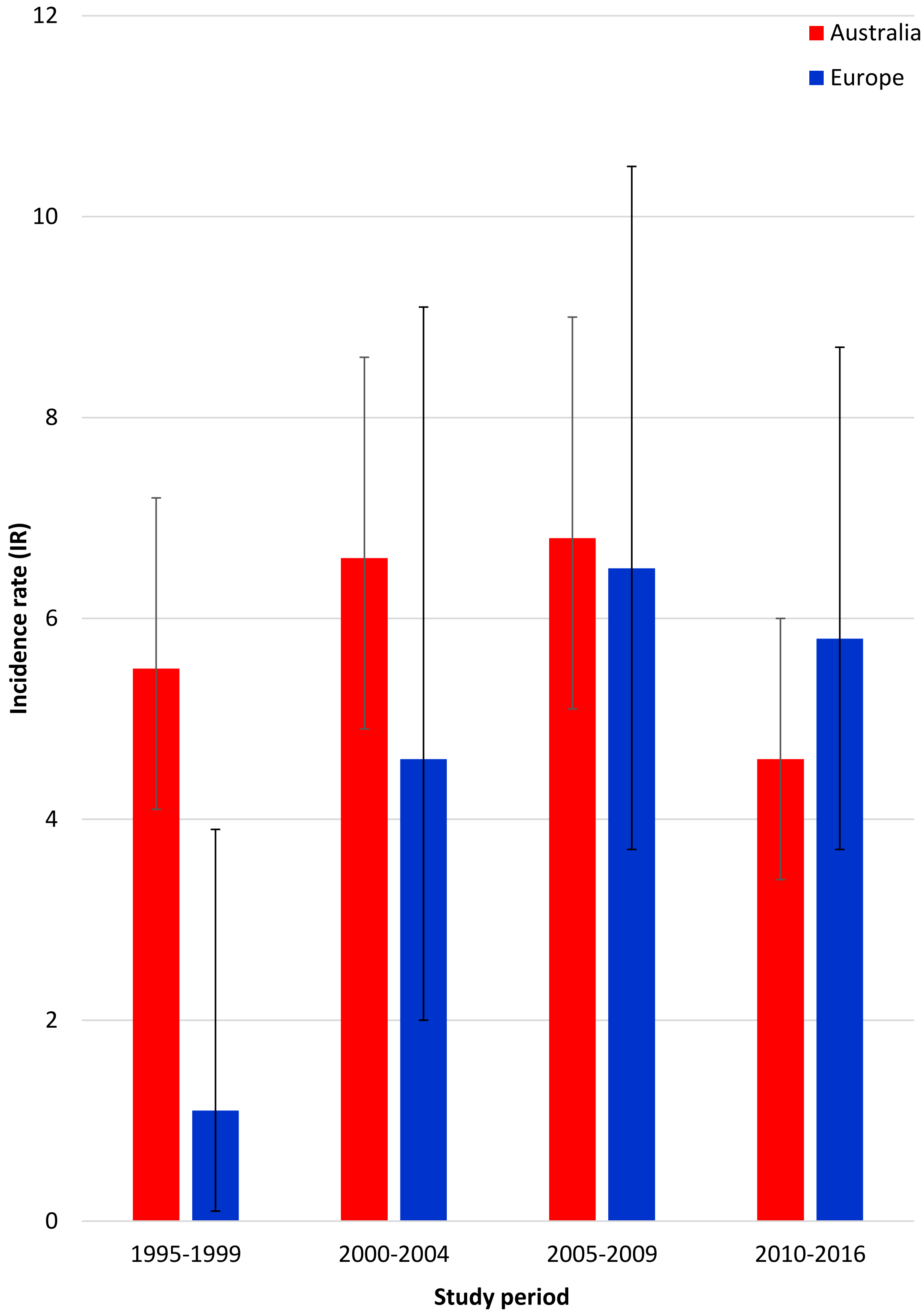

Figure 4.4: Incidence rates (cases/100 animal years) and 95% CI for lumpy jaw per study period for macropods housed at eight zoological institutions across Australia and Europe between 1st January 1995 and 28th November 2016.

Table 4.3: Adjusted incidence rate ratios (IRR) for lumpy jaw risk for macropods housed at eight zoological institutions across Australia and Europe between 1st January 1995 and 28th November 2016.

	Estimates			
Measure	**Australia**		**Europe**	
	IRR (95% CI)	***p*-value**	**IRR (95% CI)**	***p*-value**
(Intercept)	0.01 (0.00 – 0.01)	<0.001	0.00 (0.00-0.01)	<0.001
Study period				
2000-2004	1.20 (0.82 – 1.77)	0.345	4.60 (1.14 – 30.56)	0.055
2005-2009	1.10 (0.74 – 1.63)	0.633	7.39 (2.06 – 47.21)	0.008**
2010-2016	0.75 (0.50 – 1.11)	0.154	6.94 (1.96 – 44.18)	0.010**
Age group				
1-4	3.36 (1.95 – 6.31)	<0.001***	2.46 (1.02 – 7.32)	0.067
5-9	3.92 (2.22 – 7.47)	<0.001***	2.72 (0.94 – 8.93)	0.075
10+	7.63 (4.06 – 15.20)	<0.001***	7.38 (2.50 – 24.85)	<0.001***
Institution (Australia/Europe)				
Zoo A1/E1	3.88 (2.32 – 6.91)	<0.001***	1.38 (0.52 – 3.86)	0.524
Zoo A2/E2	3.27 (1.94 – 5.86)	<0.001***	1.12 (0.50 – 2.86)	0.790
Zoo A4/E3	2.09 (1.19 – 3.84)	0.013**	1.20 (0.44 – 3.45)	0.723
Sex				
Male	1.08 (0.80 – 1.44)	0.611	2.02 (1.08 – 3.83)	0.029*

Reference categories set as '1995 – 1999' for calendar period; '< 1 year' for age group; 'females' for sex; Zoo A3 for Australian institution and Zoo E4 for European institution. *$p \leq 0.05$, **$p \leq 0.01$; ***$p \leq 0.001$

4.4.4 Inter-zoo transfers

Australian region

Macropods housed in the Australian region experienced up to seven inter-zoo transfers per individual, during the study period, with a mean of 0.6 (median = 0). The odds of developing lumpy jaw increased significantly as the number of inter-zoo transfers increased (Table 4.4). Macropods that experienced only one inter-zoo transfer were 1.7 times more at risk of developing lumpy jaw than macropods that had no inter-zoo transfers (OR = 1.69, 95% CI: 1.23 - 2.32, p = < 0.001). This trend increased further to nearly 44 times the risk for those that had the greatest number of inter-zoo transfers (OR = 43.60, 95% CI: 2.08 – 915.6, p = < 0.01). The number of inter-zoo transfers could not be determined for 52 macropods.

European region

Macropods in the European region experienced up to three inter-zoo transfers per individual during the study period, with a mean of 0.4 (median = 0), although there were no macropods diagnosed with lumpy jaw that had experienced three transfers. For the European region, there were no significant relationships found between number of inter-zoo transfers and the risk of developing lumpy jaw (Table 4.4). The number of inter-zoo transfers could not be determined for three macropods.

Table 4.4: Odds ratios and 95% CI for inter-zoo transfers in relation to risk of developing lumpy jaw in macropods housed in zoological institutions in the Australian and European regions between 1st January 1995 and 28th November 2016. Reference category: macropods that had no inter-zoo transfers.

No. inter-zoo transfers	Estimates			
	Australia		Europe	
	OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**
0	-	-	-	-
1	1.69 (1.23 – 2.32)	0.001***	0.91 (0.48 – 1.71)	0.77
2	1.92 (1.19 – 3.10)	0.01**	2.4 (0.73 – 7.89)	0.14
3	3.29 (1.48 – 7.30)	0.002**	2.37 (0.10 – 59.45)	1
4	1.75 (0.20 – 12.18)	0.48	-	-
5	8.76 (1.22 – 63.02)	0.06	-	-
6	17.53 (1.57 – 195.35)	0.03*	-	-
7	43.60 (2.08 – 915.60)	0.01**	-	-

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

4.4.5 Intra-zoo transfers

Australia

The greatest number of intra-zoo transfers recorded for an individual macropod was 37, with a mean of 3.5 (median = 2) transfers per macropod for the region. The odds of developing lumpy jaw increased significantly as number of intra-zoo transfers increased (Table 4.5), with macropods that experienced two intra-zoo transfers having two and half times the risk of developing lumpy jaw than macropods with no intra-zoo transfers (OR = 2.68, 95% CI: 1.36 – 5.30, p = 0.003). This trend increased further to over 16 times the risk for those that had the greatest number of transfers (OR = 16.18, 95% CI: 8.73 – 29.98, p = < 0.0001). The number of intra-zoo transfers could not be determined for 73 macropods.

Europe

Macropods housed in the European region experienced up to seven intra-zoo transfers during the study period (mean = 0.4, median = 0), although macropods diagnosed with lumpy jaw were not reported to have experienced more than two internal transfers (Table 4.5). There was no significant association found between the risk of developing lumpy jaw and the number of intra-zoo transfers for macropods housed in the European region. The number of intra-zoo transfers could not be determined for 53 macropods.

Table 4.5: Odds ratios for intra-zoo transfers in relation to risk of developing lumpy jaw in macropods housed in zoological institutions in the Australian and European regions between 1st January 1995 and 28th November 2016. Reference category: macropods that had no intra-zoo transfers.

	Estimates			
No. intra-zoo transfers	**Australia**		**Europe**	
	OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**
0	-	-	-	-
1	1.58 (0.78 – 3.22)	0.20	1.67 (0.82 – 3.39)	0.15
2	2.68 (1.36 – 5.30)	0.003**	0.83 (0.24 – 2.88)	1
3	4.07 (2.06 – 8.04)	<0.0001***	1.09 (0.06 – 21.52)	1
4	6.61 (3.35 – 13.05)	<0.0001***	2.53 (0.10 – 63.56)	1
5	5.26 (2.45 – 11.3)	<0.0001***	-	-
6 - 10	9.93 (5.57 – 17.73)	<0.0001***	-	-
11+	16.18 (8.73 – 29.98)	<0.0001***	-	-

**$p \le 0.01$, ***$p \le 0.001$

4.4.6 Outcome (first incidence of disease)

The overall mortality rate for initial cases of lumpy jaw was 46.6% (n = 129/277) for both regions combined. Recurrence was common, to a greater extent in Australia, with more than one third of Australian-housed macropods experiencing at least a second occurrence of the disease (33.9%) (Figure 4.5). A large proportion of recurrent cases eventually succumbed to the disease (Australia 53.9%, n = 41/76; Europe 42.9% n = 3/7). A clinical resolution was achieved for nearly a quarter of European macropods (Figure 4.5), however, across both regions combined, 62.5% (n = 173/277) of macropods eventually died of the disease (Australia 61.6%, n = 138/224; Europe 66.0%, n = 35/53).

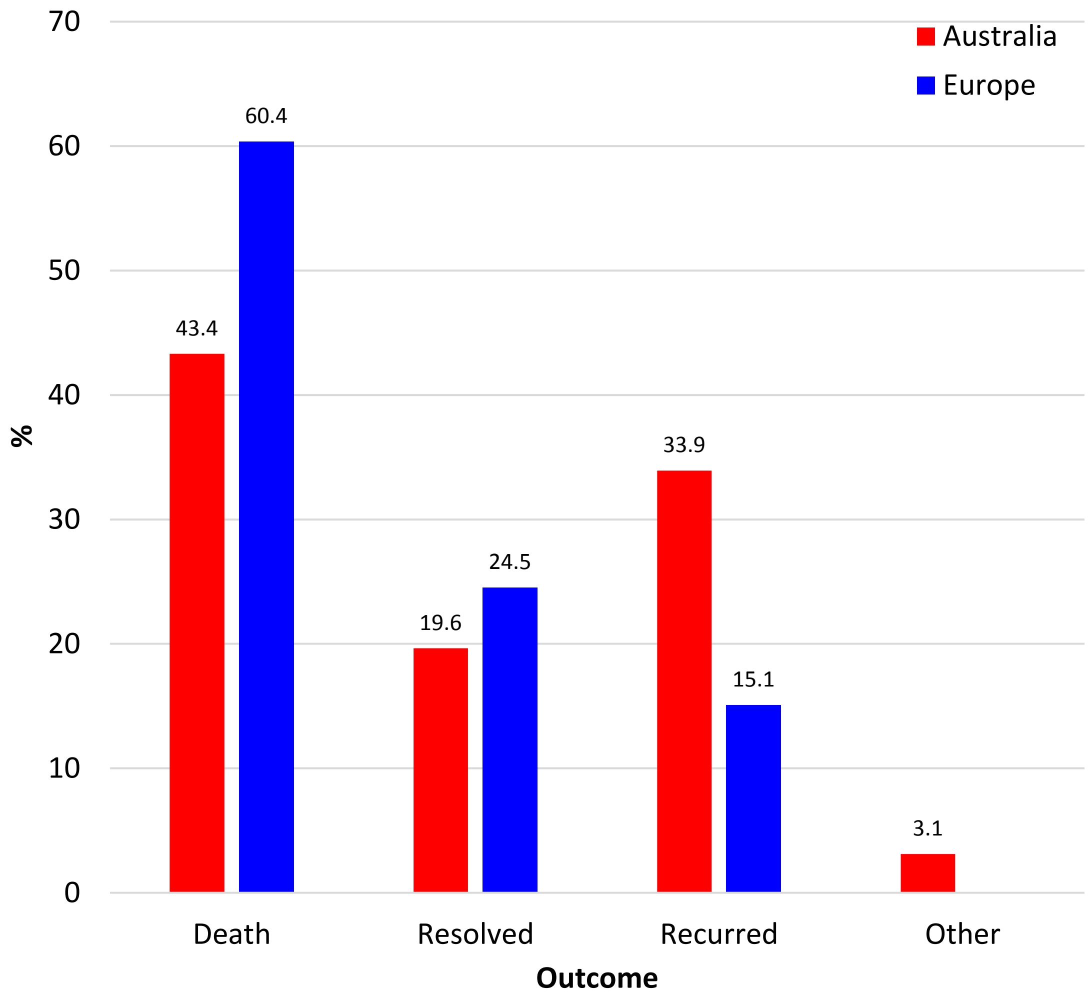

Figure 4.5: Outcome of the initial case of lumpy jaw reported in zoo records for macropods housed at eight zoological institutions across Australia and Europe between 1st January 1995 and 28th November 2016. In "Recurred" cases, 53.9% (n = 76) of Australian cases and 42.9% (n = 7) of European cases eventually died.

4.5 Discussion

This study is the first retrospective epidemiological review of lumpy jaw in captive macropods across multiple institutions in Australia and Europe, revealing a similar prevalence of the disease within each region between January 1995 to November 2016. However the IR of lumpy jaw, reflective of the risk of developing disease during an animal's lifetime in captivity, significantly increased across four European institutions from 2010 - 2016, whilst the four Australian institutions remained comparatively static for the study period. Risk factor analyses also highlighted regional differences for the significance of sex, time period, and inter- and intra-zoo transfers, which may reflect institution-specific aspects of management that are regionally derived. Overall, the outcome of lumpy jaw was poor regardless of the region in which macropods were housed, with Australia having a higher rate of recurrence of the disease in individuals that survived the initial diagnosis. Whilst the limited risk factor analyses provided biologically plausible associations, the limitations of the data and risks of confounding must be noted, and compel the use of robust prospective epidemiological study designs in future research to investigate hypotheses generated here.

4.5.1 Regional prevalence and incidence rates

Crude prevalence results across the study period indicate that the burden of lumpy jaw was similar in captive macropod populations irrespective of the geographic region where they were housed; findings that contrast with previous studies suggesting colder climates may be a risk factor (Butler, 1981; Oliphant et al., 1984; Kido et al., 2013). The prevalence reported for the Australian region at 13.8% compares to the 13.4% reported by Vogelnest and Portas (2008) although the latter was a point prevalence based on necropsy reports and therefore a biased study population. Whilst prevalence for the disease, ranging from 0 - 100% (reviewed in Chapter 1, Table 1.6, p. 30 – 31), have been reported elsewhere (Butler & Burton, 1980; Brookins et al., 2008; Vogelnest & Portas, 2008; Bakal-Weiss et al., 2010; Kido et al., 2013), these reports were often based on institutional or species-specific data and therefore are less comparable to our regional results. Importantly, the data provided by zoological institutions, where entire cohorts can be followed, and thus animal time at risk is captured, lends itself to the measure of IRs and IRRs, which more appropriately capture the level of disease and risk factors associated with the occurrence of disease for dynamic populations (Thrusfield & Christley, 2018). Whilst lumpy jaw is reported to be one of the most frequently observed diseases in captive macropods (Finnie, 1976; Arundel et al., 1977; Miller et al., 1978; Burton, 1981; Jackson, 2003; Vogelnest & Portas, 2008), the IRs calculated during this study, were seemingly low. For both Europe (4.9 cases/100 animal years) and Australia (5.7 cases/100 animal years), the IR's did not differ significantly between the regions. A review of the same dataset for other diseases of macropods and derivation of their IRs would provide the necessary perspective to determine which diseases are in fact most common for captive macropods in these regions. Further, we recommend future studies determine the IR of lumpy jaw by species, institution and/or region, to compare to results presented here.

4.5.2 Sex

Contrary to previous reports (Vogelnest & Portas, 2008), in our study the incidence of lumpy jaw differed significantly between sexes, with male macropods over two times more likely to develop the disease than females. However, this difference in risk by sex was only significant in the European region which may reflect differences in the management of male macropods (such as population structure and sex ratios), resulting in a sex-specific inability to adapt to the captive environment (Morgan & Tromborg, 2007; Mason, 2010). The provision of a captive environment that reflects the natural habitat for the species, as well as the population structure and dynamics specific to the species, is a priority for zoos and underpins individual and population health (Hosey et al., 2013; Schulte-Hostedde & Mastromonaco, 2015). Same-sex grouping is a method used for population control in zoos (Rees, 2011; Hosey et al., 2013), however does not reflect normal mob structure and may affect macropod behaviour, especially in the more gregarious species commonly led by a dominant male (Gansloßer, 1989; Dawson, 1995). Reduced access to females, and/or an increase in male competitors, may be stressful for males and increase agonistic behaviours (Gansloßer, 1989; 1995; Höhn et al., 2000; Rendle et al., 2018); risking immunosuppression which is a proposed risk factor for lumpy jaw (Blyde, 1993;

Sotohira et al., 2017a). The overall population ratio of males to females in the Australian region was close to 2:3 (643:908) (Species360, 2018), a ratio more akin to that of sex ratios reported in wild mobs, where the ratio is approximately 1:5 (red kangaroo) (Dawson, 1995). However, the overall ratio of males to females in the European region was almost 1:1 (207:209) (Species360, 2018), and if this ratio was representative of the proportions present at the zoo level, and in individual enclosures, this would reflect a sex ratio not present in wild populations.

In addition to physical and behavioural differences between the sexes, the agile wallaby and red kangaroo also show sexual dimorphism in dental development (Sharman et al., 1964; Newsome et al., 1977; Dawson, 1995), with males acquiring teeth earlier than females of the same species. Given molar progression is a hypothesised risk factor for lumpy jaw, and early development of teeth would increase the animal time at risk for an individual, there may be a sex-bias in dental development that drives the increased lifetime risk for males, and further investigation into this area should be encouraged. Another consideration is the potential relationship between macropod sex and the diet. Sexual dimorphism in body mass and size-related metabolic needs can result in differences in the diet (Mysterud, 2000) and selective feeding (Jarman, 1984; MacFarlane & Coulson, 2005; Garnick et al., 2018). In captivity, where an artificial diet is often presented, selective feeding of preferred food items may affect dental health or development. The regional bias observed in this study may therefore be associated with the sex of the macropod influencing the diet selection, and potentially from regional differences in diet presentation.

4.5.3 Age

The risk of lumpy jaw increased significantly with age, with macropods > 10 years of age more than seven times as likely to develop the disease within both regions. This result was unsurprising for a number of reasons, including the relationship between molar progression, dental disease (an age-related condition) and development of lumpy jaw (Antiabong et al., 2013a; Kido et al., 2018), and the chronicity of disease with detections often occurring in the later stages. In addition, there is reduced likelihood of detection of lumpy jaw in juveniles, due to the challenges involved in undertaking observations of the oral cavity in smaller individuals. Despite the above, we detected five cases of lumpy jaw in < 1 year old macropods; which alongside the finding of 15 juveniles with the disease by Kido et al. (2013), suggests younger individuals should not be exempt from routine examinations for this disease. The mean age for developing lumpy jaw was similar across both regions and multiple species (Australia 5.6 years [±3.6]; Europe 6.0 years [±5.1]). These mean ages differ to the findings of Kido et al. (2013), who reported a lower mean age of onset at 3.1 years (±2.1). However, both the Kido et al. (2013) study and the present study are confounded by species, which have differing lifespans and dental development, meaning the use of a relative age of onset (proportional to life expectancy), or grouping by developmental stages may be more useful. Further, given the large number of inter-zoo transfers observed in this research, cases of lumpy jaw may have been missed due to occurrence outside of our study period, or at another institution not examined in this study. Cases of lumpy jaw not captured in this study may affect the 'age of onset', as well as the incidence and prevalence calculated in our study. Incomplete records, especially during the early years of the study period, may have compounded this issue.

The relationship between ageing and lumpy jaw can be explained by multiple biologically plausible pathways; including decreasing immunocompetency (Blecha, 2000), longer exposure to environmental risk factors, likely increased lifespan in captivity (Hosey et al., 2013) and age-related dental disease as a precursor to lumpy jaw (Oz & Puleo, 2011; Antiabong et al., 2013a). Ageing leads to a number of changes to the dental arcade, such as reduced salivary flow; resulting in known precursors of lumpy jaw including gingivitis and periodontitis (Clarke, 2003; Glatt et al., 2008; Antiabong et al., 2013a). Molar progression is also age-dependent (Kirkpatrick, 1964; Dudzinski et al., 1977; Clarke, 2003; Kido et al., 2018), with several authors suggesting this is one of the main drivers of the disease (Finnie, 1976; Arundel et al., 1977; Miller et al., 1978; Kido et al., 2018). In addition, exposure to soft, high carbohydrate artificial diets has been linked to reduced tooth wear and prolonged molar progression (Finnie, 1976; Burton, 1981), which can result in oral conditions associated with lumpy jaw, such as "softened oral mucosa", gingivitis, plaque and calculus (Burton, 1981). The prolonged exposure to risk factors associated with the development of lumpy jaw, such as aspects of the diet, coupled with the continuous process of molar progression throughout life, could explain the incremental increase in risk of lumpy jaw that we observed across both regions. This forms the basis for our recommendations to increase oral examinations in older macropods to improve early detection and treatment success, and is an important area for future investigation.

4.5.4 Genus

Lumpy jaw was reported across all genera, suggesting all macropods are likely susceptible to this disease. Species within the *Wallabia, Macropus* and *Petrogale* genera were all at similar risk of disease; with only *Setonix* significantly less likely to have the disease than the other genera. *Setonix* may have a higher resilience to stressors found in captivity, such as visitor presence (Learmonth et al., 2018), although notably the majority of this species (27.9%, 85/305) were housed at Zoo A3, which also had a significantly lower burden of disease. Thus there is a risk the results for this species are confounded by institution, and future studies of the disease risk in this species would resolve the influence of institution. *Setonix* are browsers and retain premolars, a feature which subsequently blocks molar progression (Sanson, 1989; Jackson, 2003; Arman & Prideaux, 2015); therefore, we may expect to observe a lower risk of lumpy jaw in the browser group. However, this concept is not supported more broadly in our dataset, as we identified similar risks between *Macropus* (grazers) and *Wallabia* (browsers). Although smaller sample sizes at the species level, may influence our dataset and result in failure to detect a true association, from the work reported here, it appears institutional and regional management practices may be more likely to influence the incidence of lumpy jaw than the species itself.

4.5.5 Institution

Zoological institutions vary in the species they manage and the practices they use to manage them, and these practices will influence the occurrence of disease. Whilst the IR of lumpy jaw at European institutions did not differ significantly between institutions, in the Australian region one zoo (Zoo A3) had a significantly lower IR suggesting they may have managed their macropod collection differently from the other Australian institutions in this study. The Poisson regression model established that, compared to the zoo referred to as Zoo A3, the remaining zoos (Zoos A1, A2 and A4), were all at significantly greater risk of developing lumpy jaw. Overall, Zoo A1 housed the largest number of macropods over the study period, and also managed a large proportion of *Petrogale* (70.5% n = 643); a genus known to be at risk for lumpy jaw (Schultz et al., 2006; Vogelnest & Portas, 2008; Antiabong et al., 2013a). In contrast, Zoo A3's study population was nearly one-third *Setonix* (27.9% n = 305), a genus with reported low incidence of lumpy jaw (Vogelnest & Portas, 2008). Therefore, the presence of *Setonix* in greater numbers than the other institutions may have resulted in the relatively low IR for this institution. In addition to housing different genera, the captive management, housing and husbandry of macropods varied between institutions; aspects of management that all have the potential to influence the occurrence of lumpy jaw. As a result, it can be difficult to determine if institutionally based differences reflect the genera/species they house, or the management practices and environment in which those genera/species are found.

Pathways by which institutional management practices and environment can influence the health of macropods, and thus IR of lumpy jaw, include enclosure size and type (Sherwen et al., 2015; Learmonth et al., 2018; Rendle et al., 2018), and substrate provided (Ketz, 1997). Overcrowded enclosures have previously been postulated as having an associative role with lumpy jaw, through environmental loading with faecal bacteria, as well as stress (Ketz, 1997; Borland et al., 2012). However, one recent study evaluated the effect of walk-though enclosures on macropod behaviour and stress physiology for two macropod species, and found no evidence of a negative association between this particular enclosure type and the welfare of macropods (Sherwen et al., 2015). This observation may be related to the macropods' existing habituation to the number of visitors in that institution, or species-specific characteristics; as another study in a different species found that the presence of visitors in a walk-through enclosure had a 'fear provoking' effect (Learmonth et al., 2018). Although all Australian institutions in our study (when visited for data collection) had walk-through enclosures of varying size, they also exhibited differences between institutions in the number of species of macropods managed, enclosure contents and substrates. The substrate in particular may play an important role in the development of lumpy jaw; with substrates that hold moisture, such as soil and grass, being more likely to harbour pathogenic bacteria (Ketz, 1997). Environmental exposure to pathogenic bacteria has been reported to increase the risk of morbidity in horses after exposure to *Rhodococcus equi* in their environment (Takai et al., 1991). In Australia, macropods were housed on various substrates, including grass; however, the zoo with the lowest IR of lumpy jaw (Zoo A3) housed its macropods on sand, proportionally more so than the other institutions. Sand is a porous substrate, a quality that may be of benefit for reducing the ability of harmful bacteria to be maintained in the ground (Witcomb et al., 2014), which could help to explain the lower IR of lumpy jaw for this institution.

4.5.6 Intra- and inter-zoo transfers

Temporary and permanent intra-zoo transfers of macropods can occur for a variety of reasons including health concerns (movement of an individual to a hospital enclosure), the management of behaviour problems such as agonistic behaviour between males (Höhn et al., 2000), to balance population ratios (Rendle et al., 2018), or to manage captive breeding programs (Schultz et al., 2006). In addition, zoos are dynamic spaces and may often look for ways to improve enclosures for their animals (Hosey et al., 2013), which may require moving animals either temporarily or permanently to new enclosures within the grounds of the zoo. Our study found that macropods housed in Australian institutions experienced a large number of enclosure transfers (up to 37) during their lifetime, exposing them to the welfare impacts of these movements likely to be similar to those found in non-macropod species (Broom, 2003; Dembiec et al., 2004; Broom, 2005). The facilitation of pre-transportation examinations may be more likely carried out in the Australian zoos due to a greater presence of veterinary personnel. This aspect may increase both the detection and the reported incidence of lumpy jaw for the Australian region, confounding the ability to determine a true association between inter- and intra-zoo transfers and the development of lumpy jaw. Whilst the purpose of some of these moves may be to reduce stress, the movement itself is associated with stressors including pre-transportation procedures, the transport itself, confinement and adaptation to a new environment (Grandin, 1997). The resulting response may be physiological or behavioural; and fear/flight responses can result in fence-running (Jackson, 2003; Broom, 2005; Padalino et al., 2015), which may result in traumatic facial injuries that in macropods can be a precursor for infections, including lumpy jaw (Vogelnest & Portas, 2008; Padalino et al., 2015). Additionally, when lumpy jaw is present in a collection, intra-zoo transfers may also increase the risk of bacterial spread, and contaminate enclosures. Multiple transfer experiences were correlated with the development of lumpy jaw in Australian institutions, with both inter- and intra-zoo transfers being significantly associated with the disease. However, transfers to onsite veterinary facilities were included as 'intra-zoo transfers' in our study, and this could influence the results as some of these transfers would have been related to the occurrence of the disease itself, thus the risk of disease correlates with the likelihood of an internal transfer. However, the inter-zoo transfer relationship is not affected by this confounding factor, and suggests a genuine relationship exists between movements and lumpy jaw. In the Australian region, the vast distances, and subsequent extended duration of travel to other institutions, may increase the risk for, and impact of, transport associated stressors. Notably, the inter- and intra-zoo transfer effect was not significant in the European region. With only half of the European institutions having veterinary facilities onsite, this reduced the potential for macropods in European zoos to be transferred for this reason. Further studies could investigate the reasons for inter- and intra-zoo transfers, and the effect of intra-zoo transfers where movements for hospitalisation or medical management are evaluated independently of management movements. Although there may be valid conservation and welfare-related reasons for transferring animals between enclosures and zoos, to reduce the risk of developing lumpy jaw and other welfare impacts, a reduction in overall numbers of transfers, along with a review of biosecurity practices, is recommended. Additionally, to reduce the risk of oral trauma associated with handling and transport, appropriate efforts to train macropods for transportation is recommended. For example, using positive reinforcement techniques (Laule et al., 2003), by habituating macropods to transfer-related procedures, and/or through providing individuals with positive handling experiences prior to transport (Ward & Melfi, 2013).

4.5.7 Study period

Retrospective studies, including those that make use of zoo records, are an indispensable tool in the identification of disease patterns over time (Burgdorf-Moisuk et al., 2012; Griffith et al., 2013; Kido et al., 2013; Nguyen et al., 2018). As shown in domestic species (Speksnijder et al., 2017; Bailie et al., 2018), these studies can also be used to determine the efficacy of institutional changes aimed at reducing the incidence of lumpy jaw (or other disease entities); for example, changes to husbandry practices. However, retrospective studies can only be successful if records are accurate and complete, particularly for multifactorial disease entities such as lumpy jaw that have case definitions with multiple criteria. The case definition for lumpy jaw in this study aimed to capture mid to late stages of this progressive disease; however, early stages of lumpy jaw may have been missed or undiagnosed. This may have resulted in an underrepresentation of the extent to which lumpy jaw affects captive macropods across both regions. Our study found the incidence rate of lumpy jaw was stable across the entire study period for the Australian region, and had increased in the 2005 – 2016 time period in Europe. This increase may reflect changes in husbandry and management practices for this region; however, the often-dynamic nature of these practices can make evaluating these relationships difficult, particularly across institutions. This apparent increase in risk may also be related to an increase in veterinary presence in more recent years, in addition to methodological issues, as the quality and completeness of records varied between regions, institutions and time periods, and included missing and incomplete data. This issue of missing and incomplete data was more common for institutions in the European than in the Australian region, and may be the result of varied levels of veterinary support at the institutional level, to facilitate the completion of veterinary records. It is also important to note that healthy macropods in captivity may not always receive veterinary attention, therefore a medical record may never be established for these individuals.

To better understand the relationship between husbandry and management practices, and temporal changes in lumpy jaw incidence rates, would require a detailed study of each institution. Ideally, a prospective study would be used where changes are implemented in a step-wise fashion whilst controlling for other risk factors, and cohorts are monitored over reasonable time frames to detect an increase or decrease in the incidence of disease. Given the relatively low IR of lumpy jaw, the chronicity of disease, and numbers of macropods housed in some zoos, it may be difficult to effectively design prospective studies to evaluate specific risk factors related to housing and husbandry. Nonetheless, we emphasise that prospective studies would be particularly beneficial for investigating housing, husbandry and taxonomic variables that may be associated with the development of this disease. Further, we encourage zoos to contribute to the ZIMS database, and recommend its use in future health studies involving zoo animals. To improve the accuracy and increase the power of future research, we recommend that institutions have standardised case definitions and data entry protocols that include thorough, comprehensive details of health events, including lumpy jaw.

4.5.8 Outcome

For captive macropods that develop lumpy jaw, the outcome is frequently death; in this study 62.5% of the macropods that were identified as having lumpy jaw eventually succumbed to the disease. Contrary to previous studies, which have tended to be based on observations of lumpy jaw at necropsy, our results provide an overview of outcomes from lumpy jaw in live populations of captive macropods. The results from this study support previous reports that treatment for lumpy jaw is largely unrewarding (Blyde, 1999; Vogelnest & Portas, 2008). The proportion of macropod deaths following initial diagnosis for lumpy jaw in the European region (60.4% of diagnosed individuals) may indicate that either remedial treatments may not be successful, or that elective euthanasia or unassisted death occurs prior to treatment. Interestingly, clinical resolutions were greater in Europe (24.5% vs 19.2% in Australia). These figures potentially indicate a more efficacious treatment approach in the European region (examined in Chapter 5), or improvements in treatment protocols over time in that region. Uncertainties around these hypotheses are related to the small sample sizes for the region. Recurrence of lumpy jaw was expected in both regions, based on previous studies (Oliphant et al., 1984; Vogelnest & Portas, 2008); however, recurrence was more commonly observed in the Australian region. All institutions in the Australian region (but only half in the European region) had onsite veterinary services; and as discussed earlier, this difference may have facilitated treatment, rather than opting to euthanase, initial cases of lumpy jaw in the Australian region. Therefore, macropods that were treated for lumpy jaw, had a greater chance of a recurrence, thereby contributing to a higher observed rate of recurrence.

The outcome for macropods with lumpy jaw may depend on several factors including the age and health of the individual, the treatment delivered, institutional or individual veterinary belief systems relating to treatment versus euthanasia, and potentially the individuals' genetic value to the collection (Hosey et al., 2013). Additionally, changes in dental morphology related to generations of captive breeding may result in a 'genetic susceptibility' to the disease, as observed in other captive species (Clauss et al., 2007; Clauss et al., 2008; Kaiser et al., 2009). This question is worthy of further investigation, as it would assist zoos with their conservation breeding programs, and may specifically be of benefit with respect to the rarer macropod species housed in captivity. Lumpy jaw is frequently fatal, and decisions to euthanase are based on the welfare implications of this chronic and presumably painful disease. The large proportion of macropods that die as a result of the disease emphasises the need for further research into treatment efficacy and preventative measures.

4.6 Conclusion

The aims of this study were to undertake an epidemiological study of lumpy jaw in captive macropods, and identify host and environment-related risk factors associated with development of clinical disease. The risk of developing lumpy jaw was influenced by sex, age, time period and management practices, specifically increasing age and the number of inter- and intra-zoo transfers, although regional effects were often identified. Detailed examination at the institutional level is recommended to extract the specific management practices that may have resulted in differences in disease risk between institutions. Overall, this study's findings show that lumpy jaw remains an important problem for captive institutions, and efforts should be made to continue to investigate and clarify risk factors within housing and husbandry systems. Retrospective studies, including those that make use of zoo records, are an indispensable tool in the identification of disease patterns, and to provide hypotheses for prospective study designs. In addition to the need for further research, based on our findings we recommend the following to reduce the risk of developing clinical lumpy jaw: i) increase clinical examinations as macropods age, and ii) reduce the frequency of inter- and intra-zoo transfers. This new information may assist in reducing lumpy jaw mortality rates, and improve the future health and welfare of macropods in captivity.

Chapter 5

A retrospective investigation of treatment for lumpy jaw in captive macropods: A review of approaches and outcomes

5.1 Introduction

Lumpy jaw is characterised by proliferative pyogranulomatous osteomyelitis of the mandible and/or maxilla, with associated inflammation and infection (Finnie, 1976; Hartley & Sanderson, 2003; Jackson, 2003; Vogelnest & Portas, 2008; Borland et al., 2012). Treatment for lumpy jaw is often invasive and unsuccessful, with post-treatment survival low (Lewis et al., 1989). There is a lack of information on the provision of both therapeutic and prophylactic treatments for lumpy jaw. Therefore, an investigation into treatment and outcome is needed to provide an indication of the most effective treatment for this refractory disease. The current study reviewed lumpy jaw treatment and outcomes, through the retrospective analysis of zoo records for macropods housed at institutions across the Australian and European regions.

5.1.1 Dental disease in zoo animals

Dental health is of major importance to the welfare of zoo animals (Braswell, 1991; Fagan et al., 1998; Clarke, 2003; Glatt et al., 2008; Fleming & Burn, 2014). Over the past two decades advancement in the treatment of dental disease in zoo animals has been reported. This is potentially due to increased recognition of the impact of dental disease on zoo animals, in addition to knowledge transfer amongst zoological institutions, regarding the latest or most effective procedural techniques (Glatt et al., 2008; Fleming & Burn, 2014). A proactive approach to detecting dental disease has reduced dental-related mortalities in zoo animals (Braswell, 1991), as has the utilisation of various professionals working in human and veterinary dentistry, and dental training programs undertaken by zoo veterinarians (Glatt et al., 2008). Although there have been improvements in the management of dental health and disease in zoo animals over the past two decades, further research and training are still required, particularly in taxa that appear susceptible to dental disease, such as macropods.

5.1.2 Dental disease in captive macropods

Dental disease is a major health concern in captive macropods (Jackson, 2003; Antiabong et al., 2013b), however its aetiology is unknown. There is a paucity of information on the oral health of macropods (Bird et al., 2002), and knowledge of dental problems, especially in regard to lumpy jaw, are limited (Burton, 1981; Bodley et al., 2005; D. McLelland, personal communication, 17th August 2016). Lumpy jaw is the most commonly reported dental problem in macropods, yet little is known of how to successfully treat and manage this disease.

5.1.3 Diagnosis of lumpy jaw

Diagnosis of dental problems in zoo animals can be problematic, due to the challenges associated with handling and restraint (Wiggs & Lobprise, 1994). Some captive wildlife species have been trained to open their mouths using operant conditioning, which facilitates the early detection of dental problems by zoo staff (Hosey et al., 2013). However, this has yet to be reported in macropod species, most likely due to the flighty nature of many macropods and the fact that their oral cavity is very narrow, which would make visualisation of early stage dental disease in a conscious animal difficult in these species. Most dental cases in macropods require general anaesthesia for clinical examination, adding complexity and anaesthetic risk to the diagnostic investigation. Initial observation of the clinical signs of lumpy jaw, are often observed by keepers, with a diagnosis being confirmed by zoo veterinarians (Vogelnest & Portas, 2008; F. Wyss, personal commination, 13th May 2016; D. McLelland, personal communication, 17th August 2016); this is also examined in Chapter 3. Various clinical signs may be observed, however the most commonly reported is bony proliferation of the mandible and/or maxilla (Vogelnest & Portas, 2008). Standard diagnostic methods include a detailed examination of the oral cavity to identify affected teeth and the presence of lesions (Beveridge, 1934; Lewis et al., 1989; Clarke, 2003; Vogelnest & Portas, 2008). Radiography is required to determine the extent of involvement of the teeth and/or bones (Lewis et al., 1989; Vogelnest & Portas, 2008). Biopsy and/or swabbing of associated lesions in conjunction with microbial culture is often used to identify pathogenic bacteria. This enables an appropriate antibiotic/treatment regimen to be selected (Samuel, 1983; M. Lynch, personal communication, 12th November 2016). Systemic signs of disease may also be detected through abnormal haematology and biochemistry results, for example leucocytosis, neutrophilia, monocytosis, increased platelet counts, toxic changes and deviations in red blood cell indices (Burton, 1981; Hawkey et al., 1982). Elevated creatine kinase

(CK), indicative of muscle inflammation or muscle damage, and raised aspartate aminotransferase (AST), which may indicate liver or muscle inflammation, have also been reported (Hawkey et al., 1982; Vogelnest & Portas, 2008). Elevated fibrinogen levels have been reported in red-necked wallabies with confirmed lumpy jaw (Hawkey et al., 1982). Fibrinogen, an acute phase reactant, rises significantly in response to conditions that are inflammatory or result in tissue damage (Kamiya et al., 2013). As such, fibrinogen levels are considered a useful non-specific marker of inflammation, to be interpreted in conjunction with other clinical and diagnostic results.

5.1.4 Remedial treatment for lumpy jaw in macropods

Treatment for lumpy jaw is often unrewarding in macropods (Blyde, 1999; Vogelnest & Portas, 2008), and recurrence is common (Burton, 1981; Lewis et al., 1989; Vogelnest & Portas, 2008). A range of therapeutic approaches have been used, including a combination of antibiotic therapies and surgical techniques (Burton, 1981; Brookins et al., 2008; Vogelnest & Portas, 2008; Vogelnest, 2015). Surgical intervention includes the removal of mobile, infected and necrotic teeth (Lewis et al., 1989), the insertion of antibiotic impregnated polymethylmethacrylate (AIPMMA) beads (Hartley & Sanderson, 2003; Kane et al., 2017), and oral varnishes (Bakal-Weiss et al., 2010). The location and severity of the lesion(s) determines the treatment provided (Lewis et al., 1989; Kane et al., 2017). However, an important consideration in treatment outcome is the ability to provide post-operative care and appropriate housing and husbandry (Hartley & Sanderson, 2003). Some individuals do not cope with intensive therapeutic regimens and may be prone to developing exertional myopathy (Vogelnest & Portas, 2008) from frequent manual restraint and/or immobilisation. Furthermore, the efficacy of treatment options for lumpy jaw is often based on the findings from case studies where sample sizes are small (Hartley & Sanderson, 2003; Bakal-Weiss et al., 2010); therefore, outcomes may not represent the true efficacy of therapies for the condition. Treatment for lumpy jaw remains problematic, as each therapy has its own set of challenges requiring consideration and evaluation in light of the clinical signs and presentation of the macropod prior to treatment commencement.

Antibiotics

Antibiotic treatment for lumpy jaw is complex, in part due to the pathophysiology of lumpy jaw bacteria (Sotohira et al., 2017c). Bacteria adhere to bone surfaces and then colonise other areas, challenging the antibiotic's ability to reach and exert effects on associated bacterial growth (Sotohira et al., 2017c). Intensive antibiotic therapy can be offered in the initial stages of treatment, with recommended treatment regimens found in the literature (Table 5.1). The selection of an antibiotic should be based upon the results of microbial culture (Jackson, 2003; Vogelnest & Portas, 2008; M. Lynch, personal communication, 12th November 2016). The use of specific antibiotics, dose rates and delivery methods have been reported in several studies (Table 5.1). However, if culture is not undertaken, it is difficult to determine how efficacious a particular antibiotic has been, as several products may be trialled in order to reach a clinical resolution (Butler & Burton, 1980).

Delivery methods are typically based on institutional preference, the temperament of the animal receiving the antibiotic therapy, and case severity. Parenteral therapy can be administered either directly by hand or remotely via projectile syringe. Alternatively, institutions may prefer to keep their animals hospitalised until clinical resolution occurs (Wilson et al., 1980; Bakal-Weiss et al., 2010). The method of administration can affect the recovery period and outcome of treatment, due to potential stress inflicted on the animal in handling for drug delivery (Vogelnest & Portas, 2008; Staker, 2014). Regular handling/intervention for the parental delivery of antibiotics may become a source of stress (Lewis et al., 1989), as too may isolation from the mob during hospitalisation (Bakal-Weiss et al., 2010).

Table 5.1: Antibiotic products, dose rates and delivery methods, used in the treatment of lumpy jaw, as cited in the literature.

Drug	**Dose**	**Delivery method**	**Review of efficacy**
Amoxycillin (long-acting)	20 mg/kg	IM q 48 h	Bodley et al. (2005)
			Vogelnest and Portas (2008)
		q 72-94 h	Lewis et al. (1989)
Azithromycin	15 mg/kg	PO q 24 h	Plumb (2018)
Benzathine penicillin G	180,000 iu		Kilgallon et al. (2010)
Ceftiofur sodium	2 mg/kg	IM q 24 h	Bodley et al. (2005)
			Vogelnest and Portas (2008)
	14 mg		Kilgallon et al. (2010)
Clindamycin	11 mg/kg	PO bid q 12 h	Kirkwood et al. (1988)
			Bodley et al. (2005)
			Vogelnest and Portas (2008)
Enrofloxacin	100 mg for 60 days	SC q 72 h	Hartley and Sanderson (2003)
	150 mg	SC q 24 h	Hartley and Sanderson (2003)
	40 mg	PO q 24 h	Kilgallon et al. (2010)
Metronidazole	60-70 mg/kg	PO	Lewis et al. (1989)
	20 mg/kg	PO bid q 12 h	Wilson et al. (1980)
			Kirkwood et al. (1988)
			Hartley and Sanderson (2003)
Oxytetracycline	10 mg/kg	IV q 48 h	Lewis et al. (1989)
	400 mg	IM q 48 h	Hartley and Sanderson (2003)
	40 mg/kg	IM q 48 h	Butler and Burton (1980)
			Kirkwood et al. (1988)
			Lewis et al. (1989)
	20 mg/kg	q 72 hr	Vogelnest and Portas (2008)

For lumpy jaw, treatment with antibiotics alone is unlikely to be successful, often due to suboptimal concentrations being delivered to infected tissues (Hartley & Sanderson, 2003), and also because of failure to remove affected teeth or infected bone leave a nidus of infection. Prolonged use of particular antibiotics, such as oxytetracyclines (Lewis et al., 1989), can also cause diarrhoea; and in animals already challenged by the effects of lumpy jaw, debilitating side effects like diarrhoea could lead to an increasingly poor outcome. Equally, certain drug combinations such as Clavulox® (amoxicillin/clavulanic acid) (Zoetis, Rhodes, Australia) and Flagyl® (metronidazole) (Pfizer Inc., New York, USA), if given orally, may affect gastrointestinal bacteria, adversely affecting the bacteria required for healthy fermentation of ingested food (Booth & Gage, 2008; Vogelnest & Portas, 2008). The selection of antibiotics used in the treatment of lumpy jaw must be made with consideration of potential adverse effects; and ongoing observation and assessment of treatment and prognosis is recommended throughout the therapeutic process (Staker, 2014).

Topical products

Chlorhexidine is a topical antimicrobial product successfully used in the treatment of lumpy jaw in macropods (Bakal-Weiss et al., 2010). Chlorhexidine is widely used in human dentistry in the prevention and treatment of a number of oral conditions (Steinberg & Rothman, 1996; Supranoto et al., 2015). Bakal-Weiss et al. (2010) had success with using chlorhexidine varnish repeatedly painted onto the infected teeth and gingiva of *Macropus* spp. affected with lumpy jaw, in conjunction with antibiotic therapy. The slow sustained release properties of chlorhexidine were enhanced by the drying of the product in between applications. In turn, this led to extended exposure of the drug within the oral cavity, improving its efficacy (Steinberg & Friedman, 1999). Although the use of chlorhexidine is well reported for management of bacterial oral infections, such as gingivitis, in humans (Supranoto et al., 2015), the Bakal-Weiss et al. (2010) study is the only known study to report the use of chlorhexidine varnish in macropods.

The use of topical products during treatment of captive wildlife is challenging, largely due to the requirement for handling and restraint in order to apply the product, and the frequent need for additional supportive therapies. Flushing of the oral cavity and draining of oral abscesses, using a combination of topical antibiotics, irrigation fluids such as povidone-iodine, (Betadine®), has also been employed as a treatment regimen (Walker & McKinnon, 2002; Fagan et al., 2005; Vogelnest & Portas, 2008; Kane et al., 2017). However, this often needs to be provided on an ongoing basis under anaesthesia, which may not be possible and/or practical for all individuals (Fagan et al., 2005). Acute and chronic cases of lumpy jaw, which appear refractory to topical and antibiotic therapies, often require surgical intervention or euthanasia (Butler & Burton, 1980; Samuel, 1983; Kirkwood et al., 1988; Hartley & Sanderson, 2003).

Surgery

Surgical intervention is often recommended for the treatment of lumpy jaw, to facilitate a successful resolution (Butler & Burton, 1980; Hartley & Sanderson, 2003; Fagan et al., 2005; Vogelnest & Portas, 2008; Shah et al., 2016). With the support of antibiotic therapy, the debridement of infected soft tissue and bone, including the extraction of affected teeth, is commonly recommended (Braswell, 1991; Clarke, 2003; Vogelnest & Portas, 2008). Surgical procedures reported have included endodontic therapy, and accessing the tooth root apex and infected alveolar areas with apicoectomy (Kilgallon et al., 2010). Surgery can also involve the implantation procedures that provide restoration to bony deficits, after extensive debridement (Kilgallon et al., 2010), and the implantation of AIPMMA beads that directly deliver antibiotics to an infected site (Hartley & Sanderson, 2003; Kane et al., 2017). The success of AIPMMA beads is reported by Hartley and Sanderson (2003) in the treatment of lumpy jaw in a red-necked wallaby followed for 12 months. Success has also been reported in other species affected by periodontal infections, including reptiles (Divers & Lawton, 1999) and rabbits (Crossley & Aiken, 2004). Benefits associated with the use of AIPMMA beads include reduced need for repeated handling, and delivery of the required medication directly to the affected area. This targeted activity reduces the incidence of hypersensitivity reactions and common gastro-intestinal side effects, which have been reported with some systemic antibiotics (Slots & Ting, 2002). However, there are challenges with the use of AIPMMA beads, as they require the selection of an appropriate antibiotic at the time of implantation. In addition, the polymethylmethacrylate (PMMA) is not bio-absorbable; therefore, the surgical removal of beads can create further risk of infection at the surgery site (Divers & Lawton, 1999; Kane et al., 2017).

Although the recommended treatment for lumpy jaw involves the use of long-term antibiotic therapy following surgical debridement of necrotic tissues (Kirkwood et al., 1988), prognosis is guarded, and a complete resolution is often considered unlikely (Hartley & Sanderson, 2003).

5.1.5 Prophylactic treatment for lumpy jaw

Vaccination

Preventive vaccination programs are undertaken in zoos despite the lack of scientific evidence regarding their efficacy (Smith et al., 1985; Smith et al., 1986; Phillips et al., 2012). Footvax®, a vaccination produced for footrot prevention in livestock, has been proposed as a means of prevention for lumpy jaw in captive macropods (Jackson, 2003). As previously discussed, footrot can be compared to lumpy jaw, as both diseases share the presence of pathogenic bacteria, including *F. necrophorum* (and other bacteria) (Roberts & Egerton, 1969; Emery et al., 1985; Bennett et al., 2009; Zhou et al., 2009; Witcomb et al., 2014). A vaccine targeted towards *Dichelobacter nodosus*, trialled in macropods, initially appeared protective, but was later reported to be ineffective in reducing the number of cases of lumpy jaw (Blanden et al., 1987). However, vaccination trials targeted at *F. necrophorum* failed to provide evidence of efficacy against lumpy jaw (Gulland et al., 1987; Blyde, 1994). In addition to reported inefficiency of vaccines for lumpy jaw, ulcerations at the injection sites have been reported, and additionally one animal died during the observation period. However, it remains unknown if the death was associated with the vaccination (Smith et al., 1986). Therefore, currently an effective preventative treatment is unavailable.

The role of captive management in disease control

It is widely accepted that changes to husbandry procedures can reduce the presence of pathogenic bacteria associated with the development of lumpy jaw (Butler & Burton, 1980; Burton, 1981; Lewis et al., 1989; Hartley & Sanderson, 2003). Burton (1981) made recommendations for control, suggesting feeding platforms should be raised off the ground to prevent faecal contamination. Enclosure hygiene is also paramount to reduce the presence of bacteria (Witcomb et al., 2014). This includes actions such as the rotation and/or regular overhaul of substrate, and the use of dedicated feeding utensils and platforms (Burton, 1981; Jackson, 2003). However, application of these recommendations needs to take into account the size of the enclosure and overall costs; as in large macropod enclosures, the replacement or sterilisation of organic substrates may involve practical and economic challenges, discussed in Chapter 3 section 3.5.6.

Euthanasia

Euthanasia is not a treatment for lumpy jaw, however it is often an outcome of a lumpy jaw diagnosis when prognosis is poor (Vogelnest & Portas, 2008; Vogelnest, 2015). The results from radiographs assist with diagnosis and prognosis, and provide guidance as to whether treatment should be attempted (Butler & Burton, 1980). In many cases, the disease is not identified until it has reached an advanced stage, at which time treatment may no longer be considered effective. This may be due to disease severity, which is often in conjunction with secondary effects of disease, such as poor body condition associated with an inability to feed. In these cases, euthanasia may be considered the most humane option (Miller & Beighton, 1979; Lewis et al., 1989; Blyde, 1999; Jackson, 2003; Vogelnest & Portas, 2008; Vogelnest, 2015).

5.1.6 Disease recurrence and survival

Treatment for lumpy jaw is often unsuccessful, and post-therapy survival rates are low, with one study reporting only 16% of macropods surviving 12 months post-treatment (Lewis et al., 1989). The literature suggests that recurrence of disease is common (Butler & Burton, 1980; Burton, 1981; Vogelnest & Portas, 2008), with a return of lumpy jaw in the same or a novel location seen within two weeks to 18 months following cessation of antibiotic therapy (Butler & Burton, 1980; Burton, 1981; Jackson, 2003; Vogelnest & Portas, 2008).

5.2 Aims

Lumpy jaw is a chronic, and presumably painful condition that will significantly impact the welfare of an affected individual. Given the reported high mortality rates of lumpy jaw in captive macropods in zoos globally given by Lewis et al. (1989) (84%) and Kido et al. (2013) (54.4%), for example, it is important for zoo veterinarians to identify cases early, and rapidly commence treatment; prognosis is otherwise poor (Blyde, 1999; Vogelnest & Portas, 2008). An increased understanding of treatment efficacy, to shorten the duration of a lumpy jaw case, decrease case recurrence and improve overall survival, would be of great benefit to the management of this disease in captive macropods worldwide. A literature review conducted as part of this study identified a paucity of collated information regarding these issues, and it was evident that a systematic review of treatment options and outcomes was required, to provide veterinarians with a tool to critically analyse decisions concerning the treatment of lumpy jaw. As such, this research aimed to determine the treatment methods used for the clinical management and control of lumpy jaw in captive macropods in zoos across the Australian and European regions, and evaluate treatment outcome, duration and recurrence, based on the treatment provided. In addition, a statistical analysis was undertaken to investigate variables that may potentially affect the outcome of treatment for lumpy jaw, including species, age, sex, location of lesions and geographical region.

5.3 Methods

The selection of institutions involved in this study, including the methods used for the extraction of data for this retrospective study, are discussed in Chapter 2. For the purposes of this research, a case definition for lumpy jaw was developed for use in the identification of cases of the disease in zoo records; this is also reported in the General Methods chapter (Chapter 2).

5.3.1 Materials and methods

Retrospective analysis of macropod medical records, dated between 1st January 1996 and 31st December 2015, was undertaken to identify case diagnoses of lumpy jaw. Case details and clinical parameters were recorded from the initial examination, treatment and outcome for each case. Data were recorded in Microsoft® Excel 2016 for initial descriptive analysis.

Data collection

The case definition, as described in Chapter 2, was used to detect individuals that had received a case diagnosis of lumpy jaw during the retrospective study period.

Data pertaining to individual species, sex, date of birth, and institution, and details of lumpy jaw diagnosis, diagnosis date, and location of lesion(s), surgical procedures, antibiotics used, outcome and outcome date, were recorded in Microsoft® Excel 2016 for initial analysis. Unless otherwise stated, only individuals that received treatment in the form of antibiotics and/or surgery were included in these analyses.

Treatment period

Treatment duration was defined as extending from the date of diagnosis, up to and including the date of outcome (e.g. resolution, euthanasia, unassisted death/died without intervention or euthanasia). The duration of treatment for each case was calculated using a formula developed in Microsoft® Excel 2016; subtracting the 'date of outcome' from the 'date of diagnosis', to provide a figure in years. For example, an individual diagnosed on 2nd July 2010 had a case outcome date of 21st September 2010, which provided a result in days; in this case, 81 days. A second part to the formula was then created, to convert days into years, by dividing the result by 365.25 (calculating a year to be an average of 365.25 days). Therefore, the treatment period for this example is 0.2 years. The formula written into Microsoft® Excel 2016 was as follows:

= (Column outcome date – Column Diagnosis date) / 365.25

Where a case diagnosis or outcome date could not be determined, it was reported into Microsoft® Excel 2016 as 'undetermined' and no duration could be calculated for that individual.

Case recurrence

Several animals experienced more than one case occurrence of lumpy jaw during the study period, with each episode individually documented. A case recurrence would be reported if there was a clear case resolution from the previous case, and diagnosis of a new case of lumpy jaw, both described in the records. Table 5.2 explains case outcomes in detail.

Case interval period

The period of time between case recurrences was calculated using formulae in Microsoft® Excel 2016, by calculating the difference between the date of onset of the new case and the case outcome from the previous case. Where one of these dates was missing, no calculation could be made, and this individual was not included in the case interval mean calculations.

Age of onset

Age of onset was calculated using a formula created in Microsoft® Excel 2016, which calculated the difference in years between the date of first diagnosis and the animals' dates of birth. The date of diagnosis was the date the first diagnosed case of lumpy jaw occurred within the study period. If a date of birth or diagnosis was missing, no age of onset could be calculated, and the individual was excluded from analyses related to age of onset.

To enable further analysis to be performed, individuals were categorised using the mean expected longevity in captivity reported across macropod species (refer to Jackson, 2003 p. 249). There were 21 species of macropod involved in the study, all of which have differing expected longevity in captivity; therefore, the mean expected age of life expectancy of Macropodidae genera was estimated, based on the longevity data reported by Jackson (2003, p. 249). The mean life expectancy across genera was 5.9 years. For analysis, this figure was halved to provide a platform for our analyses, loosely classifying macropods as 'young' and 'old'. Individuals were then categorised as either < 3 years (young) or > 3.1 years (old) at the time of diagnosis, and to account for the latest stage of sexual maturity in some species.

Location of lesions

The location of lumpy jaw lesions and/or the location of affected teeth were reported using the anatomical location, the mandible and/or maxilla for each case of lumpy jaw. If lesions were reported in both the maxilla and mandible, they were reported as 'both'.

5.3.2 Treatment

Animals were categorised based on the treatment they received: those that received antibiotics alone, and those that received a combination of antibiotics and surgery.

Categorisation was carried out for the initial case diagnosis, and for each subsequent case recurrence. An individual classified as an 'antibiotic' treatment case was prescribed antibiotics during the treatment period, and no surgical intervention was recorded. Those classified as 'surgically' treated cases received at least one surgical procedure; for example, treatment that involved the surgical removal of teeth, debridement, and implantation of AIPMMA beads at any time within a case of lumpy jaw. The use of use of antibiotics post-surgery is routine, however some individual records did not report the use of antibiotics, or their use could not be determined; under these circumstances, these individuals were considered as 'surgical' cases. Individuals that did not receive antibiotic or surgical treatment, for which just an outcome was recorded, were recorded as 'no treatment'. Where treatment records were undeterminable, for instance where hand-written records were illegible, sections of paper records were missing, incomplete electronic records, individuals were recorded as 'undetermined'.

Antibiotics prescribed for each case and case recurrence were recorded and categorised into their respective classes prior to analysis. The number of cases for each antibiotic were calculated to provide an indication of the most commonly used product per region.

Surgery was recorded if the records indicated that, whilst under general anaesthesia, tooth extraction, debridement, curetting, apicoectomy and/or the implantation of AIPMMA beads was undertaken. As animals at some institutions were routinely anaesthetised for oral examination, it was presumed (if the purpose was not recorded), that removal of calculus and/or dental plaque was undertaken. Therefore, for the purpose of this study, general oral hygiene procedures/teeth cleaning were not considered as a surgical procedure. In addition, oral varnishes, gels and washes were not recorded, as they were frequently included in routine oral surgery treatment regimens and were not always recorded in the records.

Outcomes of treatment for lumpy jaw

An outcome was the absolute result of treatment for lumpy jaw, and an outcome was provided for each case and case recurrence. Outcomes were categorised as per the findings reported in electronic and paper-based medical, necropsy and husbandry records (Table 5.2).

Table 5.2: Outcome categories as per findings in clinical and/or husbandry records.

Outcome Category	**Explanation**
Died	Animal died without assistance (without euthanasia), where records indicated that death was associated with lumpy jaw.
Euthanased	Animal euthanased due to lumpy jaw.
Resolved	Complete resolution reported in the records.
Presumed resolved	Animals did not have a clearly defined resolution of lumpy jaw, and where there was no further evidence of ongoing oral disease and/or treatment indicated in the future records, it was presumed the case was resolved. N.B. There would be no outcome date reported for these animals.
Recurred	After a complete resolution of disease, as indicated in early records, lumpy jaw recurred in either the same or different location in or around the oral cavity.
Other	Animals were transferred to another establishment during treatment or died as a result of another cause during a period of treatment.
Undetermined	If no clear outcome could be deciphered from medical, necropsy and husbandry records, animals were recorded as ‘undetermined’. N.B. There would be no outcome date reported for these animals.

5.3.3 Statistical analysis

Data were entered in Microsoft® Excel 2016. Descriptive statistics were used initially to explore the data and to observe which treatment option/antibiotic was the most commonly used. Microsoft® Excel was also used to calculate the duration of treatment, interval periods between episodes and results for treatment outcome, location of lesions, and preference for types of antibiotic.

Statistical analysis was performed using SPSS® (IBM® Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) and Epitools (Sergeant, 2018). SPSS® was used to undertake cross-tabulation and Mann-Whitney *U* tests for differences between treatment durations, and differences between case interval periods; and Epitools (Sergeant, 2018) was used to calculate the odds ratios and 95% CI and *p*-values for the outcome of treatment. Measures of difference for categorical data, which included geographical region, sex, age and lesion location, were assessed using the chi-square test where samples sizes were > 5, as calculated in SPSS®. Where sample size in a single category was < 5, the two-tailed Fisher's exact test was used.

5.4 Results

5.4.1 Overview of treatment

Of the macropods diagnosed with lumpy jaw, 77.3% (214/277) of individuals received treatment for the condition. From these individuals, 384 cases and case recurrences of lumpy jaw were reported across both geographical regions; 324 in Australia and 60 in Europe. Treatment administered to macropods diagnosed with lumpy jaw included antibiotic therapy alone, or a combination of one or more surgical techniques with concurrent antibiotics. In the Australian region, lumpy jaw treatment more commonly involved surgical intervention, whereas in the European region, cases were more frequently treated with antibiotics alone (Figure 5.1). A total of 19.5% (75/384) of cases did not receive treatment after a diagnosis of lumpy jaw, and 0.8% (3/384) had undetermined treatment.

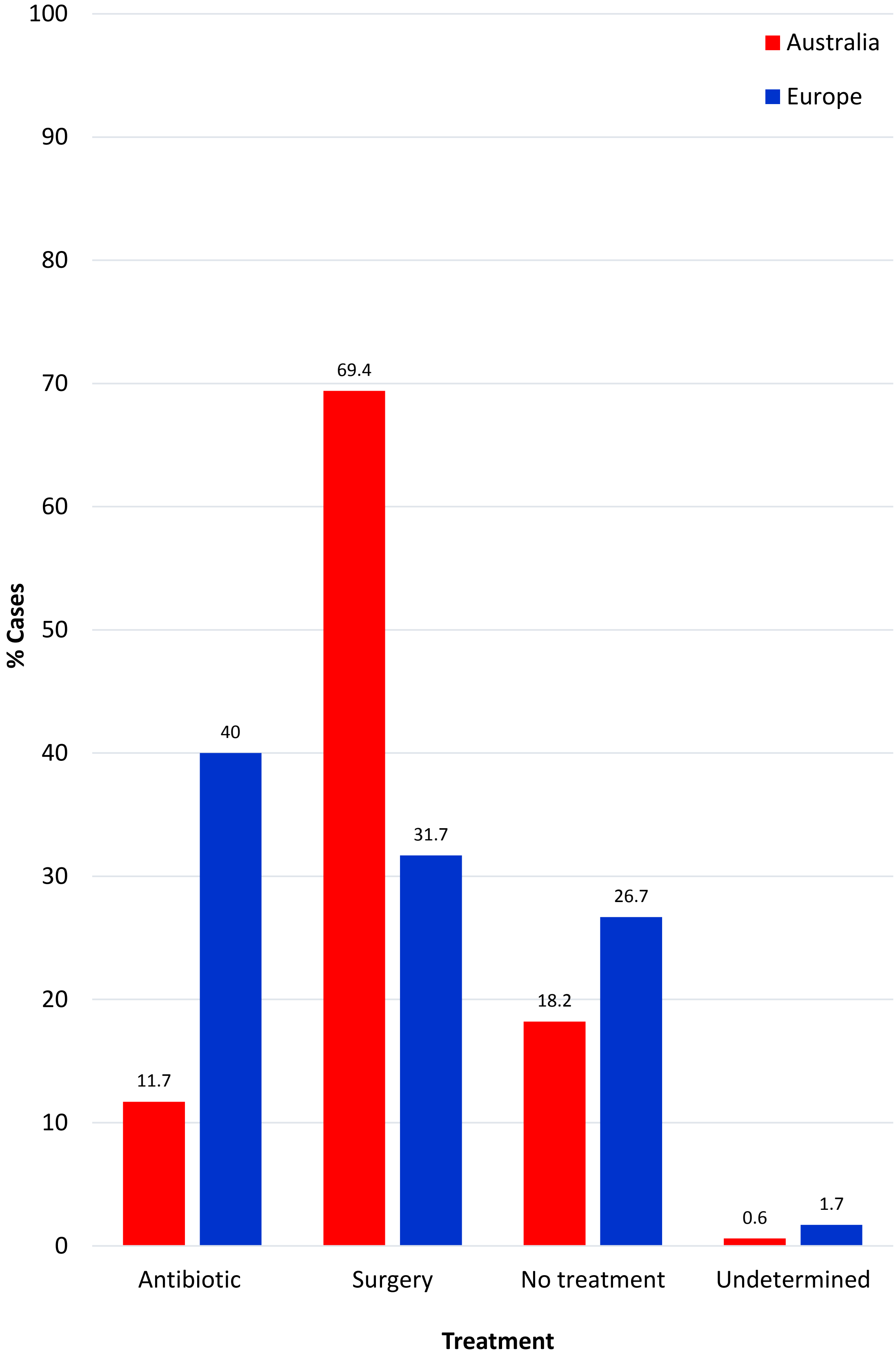

Figure 5.1: Treatment approaches used for Australian and European-housed macropods diagnosed with lumpy jaw (1st January 1996 to 31st December 2015).

The method of treatment varied with case occurrence. In both the Australian and European regions, fewer recurrent cases involved surgical procedures compared to initial cases treated. There was also an increase in cases treated with antibiotics alone in recurrent cases compared to initial cases treated (Table 5.3).

Table 5.3: Review of initial and recurrent treatment approaches used for Australian and European-housed macropods diagnosed with lumpy jaw (1st January 1996 to 31st December 2015).

		Treatment approach			
Region	**Case recurrence (n)**	**Antibiotic only %**	**Antibiotic and surgery[a] % (n)**	**No treatment %**	**ud %**
Combined regions	Initial case (277)	13.7% (38)	63.5% (176)	22% (61)	0.7% (2)
	Case recurrence (107)	22.4% (24)	63.6% (68)	13.1% (14)	0.9% (1)
Australia	Initial case (224)	8.5% (19)	71% (159)	20.1% (45)	0.4% (1)
	Case recurrence (100)	19% (19)	66% (66)	14% (14)	1% (1)
Europe	Initial case (53)	35.8% (19)	32.1% (17)	30.2% (16)	1.9% (1)
	Case recurrence (7)	71.4% (5)	28.6% (2)		

ud = treatment undeterminable. [a] Includes macropods where surgery was performed but no antibiotic was recorded (n = 7).

5.4.2 Treatment approach

Antibiotics

There were 26 antibiotics, from 11 classes (Table 5.4) used in the treatment of lumpy jaw in macropods housed across both regions. In the Australian region a total of 11.7% (38/324) cases of lumpy jaw were treated with antibiotics alone, whilst 41.7% (25/60) cases were treated using this method in the European region.

A single antibiotic was used in 48.8% (124/254) of Australian cases of lumpy jaw and 60.5% (26/43) of European cases. More than one antibiotic was used in 51.2% (130/254) of Australian cases and 39.5% (17/43) European cases.

Table 5.4: Antibiotics used in the treatment of cases of lumpy jaw in macropods housed in zoological institutions across Australia and Europe (1st January 1996 to 31st December 2015).

Class	Name	Trade name	Combined % cases (x/297)	Australia % cases (x/254)	Europe % cases (x/43)
Aminoglycosides	Gentamicin	-	3 (9)	1.2 (3)	14.0 (6)
	Tobramycin	-	0.3 (1)	-	2.3 (1)
B-lactam antibiotics	Amoxicillin	Moxylan®	13.1 (39)	13.8 (35)	9.3 (4)
Penicillins	Amoxicillin/Clavulanic acid	Clavulox®, Augmentin®	19.9 (59)	15 (38)	48.8 (21)
	Ampicillin	-	1 (3)	0.4 (1)	4.7 (2)
	Benzyl penicillin	-	2 (6)	2.0 (5)	2.3 (1)
	Carboxypenicillin	Penicillin G®	0.3 (1)	0.4 (1)	
	Procaine penicillin/ benzathine penicillin	Ticarcillin®	11.4 (34)	12.2 (31)	7.0 (3)
	Other penicillin	Norocillin®, Benacillin, Duplocillin®	10.8 (32)	-	-
		-		12.6 (32)	-
Cephalosporins	Ceftazidime	-	0.3 (1)	0.4 (1)	-
	Ceftiofur	-	3 (9)	2.8 (7)	4.7 (2)
	Cephalexin	-	0.7 (2)	0.4 (1)	2.3 (1)
Phenicols	Florfenicol		0.7 (2)	0.8 (2)	
Fluroquinolones	Enrofloxacin	Baytril®	6.7 (20)	4.7 (12)	18.6 (8)
	Marbofloxacin	-	1.7 (5)	0.4 (1)	9.4 (4)
Imidazoles	Metronidazole	Flagyl®	1.3 (4)	1.2 (3)	2.3 (1)
Lincosamides	Clindamycin	-	25.3 (75)	25.2 (64)	25.6 (11)
	Lincomycin	-	0.3 (1)		2.3 (1)
Macrolides	Tulathromycin	Draxxin®	0.3 (1)	0.4 (1)	
Sulfonamides	Trimethoprim	-	1 (3)	0.8 (2)	2.3 (1)
	Trimethoprim/Sulfadioxine	Tribrissen®	0.3 (1)	0.4 (1)	-
Tetracyclines	Doxycycline	-	1.3 (4)	1.6 (4)	-
	Oxytetracycline	-	31.3 (93)	35.4 (90)	7.0 (3)
	Tetracycline	-	1 (3)	1.2 (3)	-
Combined	Streptomycin/ dihydrostremptomycin	Penstrep®	0.3 (1)	0.4 (1)	-
	Procaine penicillin/ dihydrostreptomycin	Duplocillin®	0.7 (2)	0.4 (1)	2.3 (1)
	Lincomycin/spectinomycin	Lincospectin®	0.7 (2)	0.4 (1)	2.3 (1)

Surgery

Of the macropods receiving treatment across the Australian and European regions, surgical procedures were undertaken in the treatment of 63.3% (244/306) of lumpy jaw cases and case recurrences (Table 5.5). Surgical procedures included (singularly or in conjunction with other surgical methods): tooth extraction, reported in 87.7% (214/244) of cases and case recurrences; surgical debridement of affected tissue(s) in 50% (122/244) of cases; AIPMMA beads implanted into the site in 3.3% (8/244) of cases; and endodontic (root canal) treatment carried out in 1.2% (3/244) cases. A combination of two or more surgical procedures was undertaken in the treatment of 42.2% (103/244) of lumpy jaw cases across the Australian and European regions. Debridement in association with tooth extraction was the most common mixed method surgical procedure used in 92.2% (95/103) cases of lumpy jaw across both regions. Antibiotic impregnated beads were used in combination with other procedures in 6% (6/100) of cases (Table 5.5).

Table 5.5: Percentage of macropods housed in Australian and European zoo treated for lumpy jaw using a range of surgical methods (1st January 1996 to 31st December 2015).

		Surgical procedure % cases				
Region	**Case recurrence**	**Tooth extraction**	**Antibiotic impregnated beads**	**Debridement**	**Endodontics/Root canal**	**Mixed methods**
Combined regions	Initial (176[a])	44.3% (78/176)	1.1% (2/176)	8.5% (15/176)	0.6% (1/176)	44.9% (79/176)
	Case recurrence (68)	54.4% (37/68)	-	10.3% (7/68)	-	35.3% (24/68)
Australia	Initial (159)	42.8% (68/159)	-	8.2% (13/159)	0.6% (1/159)	48.4% (77/159)
	Case recurrence (66)	54.5% (36/66)	-	10.6% (7/66)	-	34.8% (23/66)
Europe	Initial (17[a])	58.8% (10/17)	11.8% (2/17)	11.8% (2/17)	-	11.8% (2/17)
	Recurrence (2)	50% (1/2)	-	-	-	50% (1/2)

Mixed methods: >two surgical procedures were incorporated in the duration of one case of lumpy jaw. [a]One individual in Europe had an undetermined surgical procedure.

The mixed methods approached most commonly included a combination of debridement of bone and tissue with tooth extraction in both the Australian and European regions (Table 5.6).

Table 5.6: Combination of mixed-method surgical procedures carried out in the treatment of lumpy jaw in macropods housed in zoological institutions across the Australian and European regions (1st January 1996 to 31st December 2015).

Region (Total cases)	Mixed methods (%)					
	Antibiotic impregnated beads / debridement	**Antibiotic impregnated beads / debridement / tooth extraction**	**Antibiotic impregnated beads / tooth extraction**	**Debridement / endodontics / root canal**	**Debridement / tooth extraction**	**Endodontic / root canal / tooth extraction**
Australia (100)	2% (2/100)	3% (3/100)	1% (1/100)	1% (1/100)	92% (92/100)	1% (1/100)
Europe (3)	-	-	-	-	100% (3/3)	-

5.4.3 Outcome per treatment method

Collective data from both the Australian and European regions identified that of those macropods treated for lumpy jaw, 34.3% died (71/207), 26.1% (54/207) experienced clinical resolution and 39.6% (82/207) had a clinical recurrence of disease. Of those individuals which experienced clinical recurrence, 53.7% (44/82) died following further treatment.

Across combined regions (Australia and Europe), death was the leading outcome for macropods treated with antibiotics alone, with 50% of individuals having an unassisted death, or being euthanased as a result of lumpy jaw (Figure 5.2). Recurrence of the disease was more commonly observed in those treated with surgical intervention (43.8%) across both regions combined. During the treatment period, six individuals died of other causes or were transferred to other institutions and so lost to follow-up, and one had an undetermined outcome; these individuals were excluded from the study.

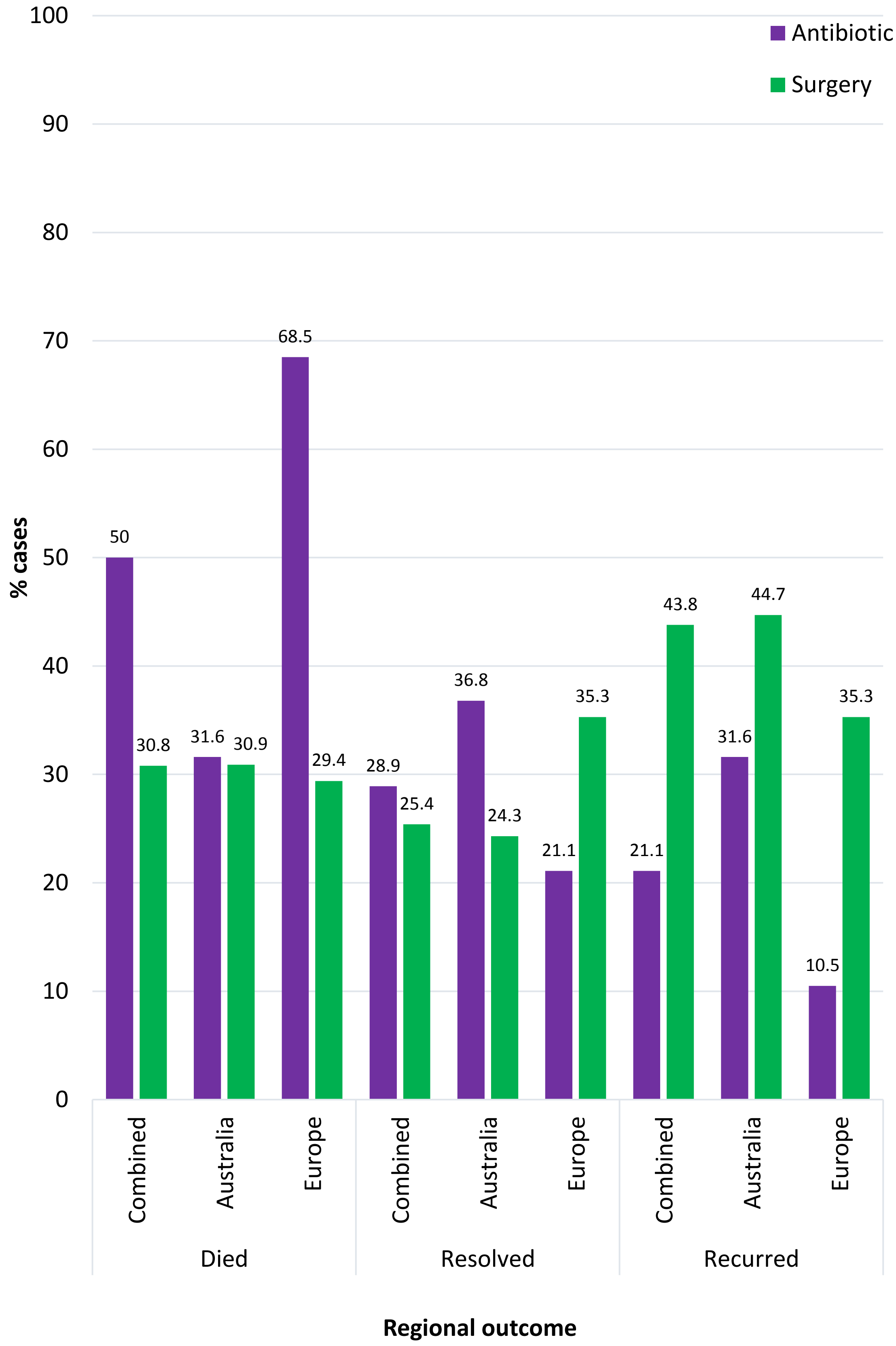

Figure 5.2: Outcome of treatment of the initial case of lumpy jaw recorded in captive macropods housed at zoological institutions in the Australian and European regions.

A Chi-square test was performed to examine the relationship between treatment approach in initial cases of lumpy jaw, and outcomes of treatment, for the combined regions (Australia and Europe). A statistically significant relationship was found between treatment type and treatment outcome for the combined regions χ^2 (2, n = 207) = 7.54, p = 0.02. Odds ratios confirmed this association, in that macropods were 3.4 times more likely to die than have a recurrence of lumpy jaw after treatment with antibiotics alone compared to surgery (OR 3.38, 95% CI: 1.38 – 8.30, p = 0.006).

Regional outcome per treatment method

A Chi-square test of independence determined there was no significant association between method of treatment and likely outcome in the Australian region (p = 0.4). However, a significant association was found between treatment for lumpy jaw and outcome in the European region χ^2 (2, n = 36) = 5.86, p = 0.05. Odds ratios indicated that macropods were nearly eight times more likely to die than have a recurrence of lumpy jaw when treated with antibiotics, this result approached statistical significance (OR 7.8, 95% CI: 1.16 – 52.35, p = 0.07). No further significant relationships were found between method of treatment and outcome in either the Australian or European regions (p > 0.05).

5.4.4 Risk factor analysis of treatment for lumpy jaw

Potential risk factors that may affect the outcome of treatment for lumpy jaw were analysed. No significant difference was found in outcome for the treatment of lumpy jaw by geographical region, sex and age (p > 0.05) (Table 5.7). A near significant association was identified between treatment type and outcome in initial cases of lumpy jaw χ^2 (4, n = 125) = 9.12, p = 0.06. On further analysis of specific outcomes, no significant difference was found between lesion location and death, case resolution and recurrences; Table 5.7 presents these findings.

Risk factor analysis of treatment for lumpy jaw - Australian region

For macropods housed in institutions within the Australian region, potential risk factors that may affect the outcome of treatment for lumpy jaw were analysed. Odds ratios found macropods with lesions in the mandibular region had a greater likelihood of achieving a clinical resolution over a recurrence OR 2.51 (95% CI: 0.99 – 6.38); a Chi-square test confirmed this was a statistically significant association χ^2 (1, n = 100) = 3.88, p = 0.05. No statistically significant relationship was found in treatment outcome when comparing between sexes or ages (Table 5.8).

Risk factor analysis of treatment for lumpy jaw - European region

For macropods housed in institutions within the European region, potential risk factors that may affect the outcome of treatment for lumpy jaw were analysed and there was no significant difference in outcome for the treatment of lumpy jaw by sex, age and lesion location (Table 5.9).

Table 5.7: Outcome of treatment for the initial case of lumpy jaw treated by geographical region, sex, age and lesion location in macropods housed within the Australian and European regions (1st January 1996 to 31st December 2015).

Characteristic (n)	**Outcome**			**Results Died / Resolved**		**Results Resolved / Recurrence**	
	Died	**Resolved % (n)**	**Recurred**	**Odds Ratio Died/Resolved (95% CI)**	***p*-value (χ^2)**	**Odds Ratio Resolved/Recurred (95% CI)**	***p*-value (χ^2)**
Region							
Australia (178)	29.8 (53)	24.7 (44)	41.6 (74)	0.67 (0.28 - 1.6)	0.36 (0.82)	0.48 (0.18 - 1.29)	0.14 (2.17)
Europe (36)	50.0 (18)	27.8 (10)	22.2 (8)				
Sex							
Male (96)	32.3 (31)	17.7 (17)	24.0 (23)	1.69 (0.8 - 3.54)	0.17 (1.92)	1.18 (0.56 - 2.49)	0.67 (0.18)
Female (181)	22.1 (40)	20.4 (37)	32.6 (59)				
Age (years)							
<3 (70)	22.9 (16)	22.9 (16)	27.1 (19)	0.75 (0.3 3- 1.7)	0.49 (0.48)	1.4 (0.64 - 3.07)	0.4 (0.72)
>3 (186)	25.8 (48)	19.4 (36)	32.3 (60)				
Lesion							
Mandible (148)	15.9 (34)	15.4 (33)	20.1 (43)	0.61 (0.24 - 1.52)	0.28 (1.16)	1.92 (0.81 - 4.54)	0.14 (2.23)
Maxilla (67)	7.9 (17)	4.7 (10)	11.7 (25)				

Table 5.8 Outcome of treatment for the initial case of lumpy jaw treated by sex, age and lesion location in the Australian macropods (1st January 1996 to 31st December 2015).

	Outcome			**Results Died / Resolved**		**Results Resolved / Recurrence**	
Characteristic (n)	**Died**	**Resolved % (x)**	**Recurred**	**Odds Ratio (95% CI)**	***p*-value (χ^2)**	**Odds Ratio (95% CI)**	***p*-value (χ^2)**
Sex							
Male	38.2	21.8	36.4				
(55)	(21)	(12)	(20)				
				1.75	0.2	1.01	0.98
Female	26.0	26.0	43.9	(0.74 - 4.14)	(1.63)	(0.44 - 2.34)	(0)
(123)	(32)	(32)	(54)				
Age (years)							
< 3	30.0	27.5	40.0				
(40)	(12)	(11)	(16)				
				0.94	0.9	1.22	0.66
> 3	36	31	55	(0.36 - 2.43)	(0.02)	(0.5 - 2.96)	(0.19)
(127)	(28.3)	(24.4)	(43.3)				
Lesion							
Mandible	30.5	29.5	35.2				
(105)	(32)	(31)	(37)				
				0.64	0.38	2.51	0.05*
Maxilla	28.3	17.4	52.2	(0.23 - 1.74)	(0.78)	(0.99 - 6.38)	(3.88)
(46)	(13)	(8)	(24)				

*$p \leq 0.05$

Table 5.9: Outcome of treatment for the initial case of lumpy jaw treated by sex, age and lesion location in the European macropods (1st January 1996 to 31st December 2015).

Characteristic (n)	Outcome			Result Died/Resolved		Result Resolved/Recurrence	
	Died	**Resolved % (n)**	**Recurred**	**OR (95% CI)**	***p*-value ($^b\chi^2$)**	**OR (95% CI)**	***p*-value (χ^2)^b**
Sex							
Male	55.6	27.8	16.7				
(18)	(10)	(5)	(3)	1.25	0.78	1.67	0.96
				(0.27 - 5.89)	(0.08)	(0.25 - 11.07)	(0)
Female	44.4	27.8	27.8				
(18)	(8)	(5)	(5)				
Age (years)							
< 3	33.3	41.7	25.0				
(12)	(4)	(5)	(3)	0.38	0.77	1.67	0.96
				(0.06 - 1.79)	(0.38)	(0.25 - 11.07)	(0)
> 3	54.5	22.7	22.7				
(22)	(12)	(5)	(5)				
Lesion							
Mandible	20.0	20.0	60.0				
(10)	(2)	(2)	(6)	0.5	1	0.17	0.56
				(0.04 - 6.68)	(0)	(0.01 - 2.98)	(0.33)
Maxilla	7.1	28.6	14.3				
(7)	(4)	(2)	(1)				

$^b\chi^2$ Yates corrected where a factor was < 5

Species

The outcome of treatment in the initial case is reported in Table 5.10 for the Australian region and Table 5.11 for the European region. Western grey kangaroos housed in the Australian region had a greater percentage of case resolutions following surgery for lumpy jaw (80%), than those housed in the European regions (37.5%). Odds ratios determined that Australian western grey kangaroos receiving surgical treatment for lumpy jaw were no more likely to have a case resolution than a recurrence than their European conspecifics (OR 4.00, 95% CI: 0.26 – 60.33, p = 0.7). One hundred percent of Australian housed red-necked wallabies died when treated for lumpy jaw using antibiotics alone, whereas there was a lower proportion of deaths in this species when treated in the same manner in the European region (87.5%). Where analyses permitted, no significant difference was found between the outcome of treatment for species housed in both the Australian region and European regions, and the treatment type ($p > 0.05$).

Age at first diagnosis (Table 5.12)

The mean age (±SD) of initial diagnosis across regions (Australia and Europe) was 5.7 years (± 3.94). Within Australia, the mean age of initial diagnosis of lumpy jaw was 5.6 (± 3.60) years, with the youngest age recorded at first diagnosis being 0.3 years or 110 days. The oldest age of diagnosis was 17.3 years. There were 18 animals from the Australian region for which age at diagnosis could not be ascertained. In Europe, the mean age of diagnosis in the European macropods was 6.0 years (± 5.14). The youngest recorded age was 0.3 years (western grey kangaroo) and the oldest was 18.9 years (red-necked wallaby). There were three individuals from the European region for which the age at diagnosis could not be ascertained.

Table 5.10: Outcome of treatment for initial cases of lumpy jaw using antibiotics alone or surgery for macropod species housed in the Australian region.

Genus	Species common name (Total no. treated)	Antibiotic % treated			Surgery % treated		
		Died	Resolved (x/n)	Recurred	Died	Resolved (x/n)	Recurred
Dendrolagus	Goodfellow's tree kangaroo (2)	-	100% (1/1)	-	-	-	100% (1/1)
	Matschie's tree kangaroo (5)	-	-	-	20% (1/5)	40% (2/5)	20% (1/5)
Lagorchestes	Spectacled hare wallaby (1)	-	-	-	100% (1/1)	-	-
Macropus	Agile wallaby (4)	-	-	-	50% (2/4)	50% (2/4)	-
	Black-striped wallaby (3)	-	-	-	66.7% (2/3)	-	33.3% (1/3)
	Common wallaroo (2)	100% (1/1)	-	-	-	100% (1/1)	-
	Eastern grey kangaroo (2)	-	100% (1/1)	-	-	-	100% (1/1)
	Red kangaroo (40)	100% (1/1)	-	-	32.4% (12/39)	16.2% (6/39)	51.4% (19/39)
	Red-necked wallaby (22)	100% (1/1)	-	-	33.3% (7/21)	14.3% (3/21)	52.4% (11/21)
	Tammar wallaby (18)	-	33.3% (1/3)	66.7% (2/3)	40% (6/15)	13.3% (2/15)	46.7% (7/15)
	Western brush wallaby (3)	-	-	-	66.7% 2/3	33.3% (1/3)	-
	Western grey kangaroo (6)	100% (1/1)	-	-	-	80% (4/5)	20% (1/5)
Onychogalea	Bridled nail-tail wallaby (1)	-	-	-	-	100% (1/1)	-
Petrogale	Black-footed rock wallaby (7)	-	-	-	14.3% (1/7)	14.3% (1/7)	71.4% (5/7)
	Brush-tailed rock wallaby (7)	-	100% (1/1)	-	16.7% (1/6)	66.7% (4/6)	16.7% (1/6)
	Yellow-footed rock wallaby[a] (38)	16.7 % (1/6)	33.3% (2/6)	50% (3/6)	12.5% (4/32)	21.9% (7/32)	50% (16/32)
Setonix	Quokka (7)	-	100% (1/1)	-	50% (3/6)	-	50% (3/6)
Thylogale	Red-legged pademelon (1)	-	-	-	100% (1/1)	-	-
	Tasmanian pademelon (3)	100% (1/1)	-	-	100% (2/2)	-	-
Wallabia	Swamp wallaby (7)	-	-	-	8.6% (2/7)	28.6% (2/7)	42.9% (3/7)

[a]Four individuals that received surgical treatment for lumpy jaw had other outcomes: death due to other causes, or they were transferred to another establishment before an outcome was determined (12.5%). One individual had an undermined outcome (3.1%).

Table 5.11: Outcome of treatment for initial cases of lumpy jaw using antibiotics alone or surgery for macropod species housed in the European region.

Genus	Species common name (Total no. treated)	Antibiotic			Surgery		
		Died	Resolved	Recurred	Died	Resolved	Recurred
		% treated with antibiotics (x/n)			% treated with surgery (x/n)		
Macropus	Agile wallaby (1)	100% (1/1)	-	-	-	-	-
	Common wallaroo (2)	100% (2/2)	-	-	-	-	-
	Red kangaroo (5)	50% (1/2)	50% (1/2)	-	-	33.3% (1/3)	66.7% (2/3)
	Red-necked wallaby (13)	87.5% (7/8)	-	12.5% (1/8)	40% (2/5)	40% (2/5)	20% (1/5)
	Western grey kangaroo (14)	33.3% (2/6)	50% (3/6)	16.7% (1/6)	25% (2/8)	37.5% (3/8)	37.5% (3/8)
Petrogale	Brush-tailed rock wallaby (1)	-	-	-	100% (1/1)	-	-

Table 5.12: Mean age of first diagnosis (±SD) of lumpy jaw for all species housed across Australian and European zoos (1st January 1996 to 31st December 2015).

Genus	**Species common name**	**Australia Age (years) (±SD) n**	**Europe Age (years) (±SD) n**
Dendrolagus	Goodfellow's tree kangaroo	9.5 (2.04) 2	-
	Matschie's tree kangaroo	13.7 (2.43) 5	-
Lagorchestes	Spectacled hare wallaby	4.6 (0.00) 1	-
Macropus	Agile wallaby	4.6 (2.71) 4	5.6 (0.00) 1
	Black-striped wallaby	3.9 (4.39) 3	-
	Common wallaroo	5.0 (2.08) 2	11.1 (2.79) 2
	Eastern grey kangaroo	5.7 (3.79) 2	-
	Red kangaroo	5.7 (3.65) 49	4.8 (3.90) 6
	Red-necked wallaby	5.9 (3.92) 34	5.5 (4.79) 20
	Tammar wallaby	5.1 (2.33) 23	-
	Western brush wallaby	7.6 (5.41) 3	-
	Western grey kangaroo	7.5 (4.32) 8	6.0 (5.22) 21
	Whiptail wallaby	6.9 (0.00) 1	-
Onychogalea	Bridled nail-tail	5.7 (0.00) 1	-
Petrogale	Black-footed rock wallaby	3.9 (2.17) 10	-
	Brush-tailed rock wallaby	4.7 (2.83) 10	3.3 (0.00) 1
	Yellow-footed rock wallaby	4.8 (2.74) 46	-
Setonix	Quokka	10.0 (3.65) 7	-
Thylogale	Tasmanian pademelon	4.5 (3.43) 4	-
	Red-necked pademelon	0.5 (0.00) 2	-
Wallabia	Swamp wallaby	5.2 (3.25) 7	5.3 (5.59) 2

Location of lesions

Collectively, 52.5% of cases of lumpy jaw (n = 202) presented with lesions in the mandible, while 23.7% (n = 91) were located in the maxilla. There were 6% of cases (n = 23) where oral lesions occurred in both the mandible and the maxilla, and 17.7% of all cases recorded (n = 68) had no lesion location recorded (Table 5.13).

Table 5.13: Location of lumpy jaw lesions and case recurrence(s) by geographical region.

		Location of oral lesion(s)			
Region	**Case (n)**	**Mandible % (n)**	**Maxilla % (n)**	**Both regions % (n)**	**Undetermined % (n)**
Australia	Total (384)	47.7% (183)	20.6% (79)	4.9% (19)	11.2% (43)
	Initial case (224)	40.1% (130)	25.4% (57)	4.0% (13)	7.4% (24)
	Case recurrence (100)	53.0% (53)	22.0% (22)	6.0% (6)	19.0% (19)
Europe	Total (60)	31.7% (19)	0.0% (12)	6.7% (4)	41.7% (25)
	Initial case (53)	32.1% (17)	18.9% (10)	5.7% (3)	43.4% (23)
	Case recurrence (7)	28.6% (2)	28.6% (2)	14.3% (1)	28.6% (2)

In the Australian region, institutions reported the recurrence of 45.3% (n = 34) of lumpy jaw lesions in a different location of the oral cavity (mandible/maxilla) than the original site of detection. A total of 36% (n = 27) were reported as having a case recurrence in the same area (mandible/maxilla) of the oral cavity.

In the European region, 28.6% (n = 2) of macropods experienced clinical recurrence in the same location within the oral cavity at first diagnosis; equal to those where recurrence occurred in a new region compared to original detection.

5.4.4 Duration of treatment

The mean (±SD) duration of treatment, taken from the date of diagnosis to the date of outcome, collectively for all macropods involved in this study was 0.14 years (± 0.01) (median 0.08) or 52 days. The maximum duration period for the delivery of treatment was 1.2 years or 438 days. Results for the duration of treatment, by treatment approach and region, are presented in Table 5.14.

Region (Table 5.15)

In the Australian region, the overall mean duration of treatment was 0.16 (± 0.01) years (median 0.08) (59 days). The longest duration of treatment for lumpy jaw in Australian housed macropods was 1.23 years (448 days). The duration of treatment was unable to be determined for 31 individuals due to incomplete records, therefore these animals were excluded from the analyses.

In the European region, the mean duration of treatment for lumpy jaw was 0.11 (±0.02) years (median 0.04) (40 days). The maximum duration of treatment in European macropods was 1.01 years (370 days). The duration of treatment could not be determined for eight individuals, therefore these animals were excluded from the analyses.

Table 5.14: Mean duration of treatment in years (±SD) (days) by treatment type/case of lumpy jaw, for Australian and European housed macropods.

	Treatment Approach		**Result**
Region	**Antibiotics** **Mean duration years (±SD)** **(days)**	**Surgery** **Mean duration years (±SD)** **(Days)**	***p*-value** ***U* value**
Combined regions	0.12 (0.16) (44 days)	0.20 (0.25) (74 days)	0.001*** 4062.5
Australia	0.09 (0.09) (33 days)	0.21 (0.26) (76 days)	0.001*** 2182.0
Europe	0.17 (0.23) (61 days)	0.09 (0.10) (32 days)	0.96 138.0

***$p \leq 0.001$

Case recurrence

Overall the duration of treatment for lumpy jaw in macropods across both regions was significantly shorter in the initial case than in recurrent cases (p = 0.03). A significant result was also determined for the European macropods (p = 0.05). There was no significant difference in mean duration of treatment and case occurrence in macropods housed in the Australian region (p > 0.05) (Table 5.15).

For initial cases of lumpy jaw, there was no significant difference in mean duration of treatment by region (Australia or Europe), and this lack of difference held for both treatment types: antibiotics (p = 0.57) and surgery (p = 0.08). In addition, there was no significant difference determined in the mean duration of treatment in recurrent cases by region (Australia or Europe); a non-significant result was found for both treatment types: antibiotics (p = 0.27) and surgery (p = 0.30).

Table 5.15: Mean duration of treatment for initial and case recurrences of lumpy jaw, in years (±SD), by geographic region.

Region	**Initial case Mean years (±SD) (days)**	**n**	**Case recurrence Mean years (±SD) (days)**	**n**	***p*-value *U***
Combined	0.14 (0.21) (53 days)	242	0.19 (0.27) (69 days)	102	0.03* 10540.5
Australia	0.14 (0.21) (53 days)	196	0.22 (0.27) (69 days)	97	0.14 8509.0
Europe	0.10 (0.17) (37 days)	47	0.19 (0.13) (69 days)	5	0.05* 54.5

*$p \leq 0.05$

Period of quiescence

The mean period of quiescence between an initial case of lumpy jaw that was treated and the first case recurrence for the combined dataset was 1.2 years (± 1.13, n = 72). The mean period of quiescence between an initial case of lumpy jaw that was treated and the first case recurrence within the Australian region was 1.23 years (± 1.25, n = 68) and within the European region it was 0.38 years (± 0.22, n = 4).

Collectively, macropods in this study that were treated with antibiotics alone had a mean period of quiescence between case resolution and case recurrence of 1.4 years (± 1.19, n = 8) or 511 days. For those treated with surgery, the mean period of quiescence was 1.16 years (± 1.13, n = 64) or 424 days. A Mann-Whitney U test found there was no significant difference in the period of quiescence between the initial case outcome and the first case recurrence, for the two treatment approaches ($p > 0.05$).

5.5 Discussion

The treatment of lumpy jaw in captive macropods remains a challenge for veterinarians, despite advances in antibiotic therapy and surgical techniques. Results from this longitudinal retrospective cohort study (1996 – 2015) identified that the treatment for lumpy jaw follows recommendations found in the literature (Blanden et al., 1987; Vogelnest & Portas, 2008; Bakal-Weiss et al., 2010; Vogelnest, 2015). Across both regions (Australia and Europe), a greater percentage of initial lumpy jaw cases were treated using a combination of surgery and antibiotics (63.5%), than were treated with antibiotics alone (13.5%). Treatment for the condition carried out in the Australian region showed a notable preference for surgical intervention in initial cases (71%), whereas macropods in the European region were most frequently treated exclusively with antibiotics (35.8%). Macropod death was the most common outcome, significantly so in those treated with antibiotics alone ($p = 0.006$). Risk factor analyses found no association between lumpy jaw and sex, age and lesion location, however treatment type (antibiotics alone and surgery) and geographical region (Australia, Europe) both had a significant influence on the duration of treatment. This research contributes to the literature on the management of lumpy jaw in macropods by providing empirical evidence of the likely outcomes of treatment for this refractory disease.

5.5.1 Diagnosis

It is interesting to note that a review of zoo records identified that diagnosis of lumpy jaw, throughout the retrospective period, was consistent with methods previously described by Burton (1981), Jackson (2003) and Vogelnest and Portas (2008). These methods included monitoring for clinical signs to observe visible lumps to the jaw, changes in feeding behaviour, hyperptyalism, blepharospasm, and ocular and nasal discharge, with the condition confirmed through clinical examination, radiography, biopsy and/or swabbing of lesions for microbial culture. Not all methods of diagnosis were used in all cases; the ability to diagnose clinical signs of lumpy jaw was dependent on the resources available at each institution. Some of the European institutions did not have resident veterinarians or facilities available for undertaking confirmatory diagnostic methods, such as radiography. Therefore, diagnosis in some institutions would be restricted to the observation of clinical signs, such as observing changes in feeding behaviour. The observation of changes in behaviour could be considered as more subjective, and reliant upon the experience of the observer, which will vary between institutions. In addition, diagnostic methods may have changed over time, affecting the number of cases diagnosed. In some cases, lumpy jaw was initially detected in necropsy reports as either an initial finding after the death or following euthanasia of the individual.

5.5.2 Evaluation of treatments

Macropods receiving treatment for lumpy jaw received one of two approaches: antibiotic therapies alone, or surgery in conjunction with supportive antibiotics. Of those macropods diagnosed with lumpy jaw across both the Australian and European regions, between 1st January 1996 to 31st December 2015, 72.3% received treatment for the condition. Knowledge of the most effective treatment approach to reduce the case severity of lumpy jaw, or its duration, is beneficial to the zoo veterinarian. However, not all macropods diagnosed with lumpy jaw received treatment. Animals that did not receive treatment were recorded as either an unassisted death, or having been euthanased due to the condition. In euthanased individuals, it could be assumed that treatment would not have been beneficial in providing a positive outcome; however, the case severity was not detectable from veterinary records, therefore other aspects may have affected the decision to euthanase. The decision to perform euthanasia on zoo animals, regardless of taxa, is complex, and involves not only consideration of health, disease and mob dynamics, but also those of a financial and ethical nature (Föllmi et al., 2007). The presence of physical resources, and suitably trained personnel to treat the condition and manage the aftercare, are key to the health, welfare and outcome of macropods with lumpy jaw. There are, however, additional considerations, including the age of the animal affected, and its genetic value to the collection; whereby its removal may benefit the genetic viability of breeding animals (Rees, 2011). To allow for genetic diversity in a population and avoid saturation through inbreeding, schemes such as Maximum Avoidance Inbreeding (MAI) schemes (Wright, 1921; Princée, 1998), are in place to preserve a healthy genetic population in captivity. They function by the removal of individuals of a particular generation or sex, to enable others to breed, increasing the demographic variation of ex-situ populations (Leus et al., 2011; Princée, 2016). All institutions involved in this research housed macropods that did not receive any treatment for lumpy jaw. It is difficult to make assumptions as to why institutions elected not to treat macropods affected with lumpy jaw. However, a small number of animals (n = 3) that had no record of surgical or medicinal treatment, had a complete resolution. Given the known pathology of lumpy jaw, it would seem unlikely that the case diagnosed was a true case of the disease; or possibly treatment was administered but not recorded.

Treatment for lumpy jaw has not changed markedly over the last 20 years, with the repeated appearance of two therapeutic approaches observed in zoo records: antibiotics alone, or a combination of surgery and antibiotics. Treatment approaches varied across the two regions, with more cases of lumpy jaw being treated with surgical intervention in the Australian region (69.4%), and institutions in the European region demonstrating a greater use of antibiotics alone (40%). The range of surgical procedures carried out in both regions was similar, as too were the types of antibiotics prescribed for lumpy jaw.

Antibiotics

A range of antibiotics from several classes were used in the treatment of lumpy jaw over the 20-year period, many of which are recommended in the literature (Table 5.1). Collectively, across both regions, the use of antibiotics alone in the treatment of initial cases was low (13.7%), although in subsequent case recurrences there was an increase in use of this treatment (22.4%). Treatment for lumpy jaw by means of antibiotics alone was more commonly observed in the European institutions, with nearly four times the percentage of initial cases (35.8%) being treated in this way compared to the Australian region (8.5%).

The use of antibiotics alone in the treatment of lumpy jaw has repeatedly been demonstrated to be unsuccessful (Samuel, 1983; Kirkwood et al., 1988; Hartley & Sanderson, 2003). In support of these findings, results from this study found that individuals in the Australian region treated with antibiotics alone reported a mortality rate of 31.6%. However, the number of animals treated with antibiotics alone in the Australian region was low (n = 19). In contrast, the percentage of macropods that died in the European region was more than twice that of those in the Australian region (68.4%), yet similar antibiotic products were used. The selection of an appropriate antibiotic is typically based on results of biopsy and/or cytology of lesions and microbial culture (Samuel, 1983; Jackson, 2003). However, the swabs taken may be contaminated with pathogens or commensal bacteria from areas overlying the focus of the lumpy jaw lesion, leading to the isolation of non-related or synergistic pathogens, not necessarily the causative pathogen. This would provide an inaccurate indication of causative bacteria, reducing the efficacy of antibiotic selection. Microbial culture was routinely carried out at many of the institutions involved in this study (S. Vitali, personal communication, 23rd November, 2015; C. Wenker, 2016, personal communication, 13th May 2016; G. Sayers, personal communication, 26th May 2016; L. Vogelnest, 2016, personal communication, 10th August 2016; D. McLelland, personal communication, 17th August 2016; M. Lynch, personal communication, 12th November 2016); however, analysis of culture results in relation to antibiotic selection and outcome could not be undertaken, due to inadequate record keeping. In future, a more detailed investigation of the bacterial species isolated and antibiotics selected would benefit current knowledge of antibiotic efficacy in cases of lumpy jaw. In addition, this information may assist in the development of an effective vaccination program, similar to that used to prevent footrot in sheep (Witcomb et al., 2014); a bacterial disease likened to lumpy jaw.

There were 26 different antibiotics used in the treatment of lumpy jaw, across both regions, presented in Table 5.4, including the products used for individuals receiving surgical intervention. Across both the Australian and European regions, the top five most commonly used antibiotics for the treatment of lumpy jaw were oxytetracycline, clindamycin, amoxicillin/clavulanic acid, amoxicillin and procaine penicillin/benzathine penicillin. The antibiotic used most commonly in the European region was amoxicillin/clavulanic acid (48.8% of cases). This product extends the spectrum of activity of amoxicillin to include β-lactamase-producing species (Tancawan et al., 2015), including some species found in culture from lumpy jaw lesions, for example, *Staphylococcus* (Samuel, 1983), *Escherichia* (Antiabong et al., 2013a) and *Fusobacterium* (Samuel, 1983; Vogelnest & Portas, 2008; Antiabong et al., 2013b). The choice of antibiotic is determined by bacterial culture, antibiotic sensitivity, tissue penetration and pharmacokinetics, and also by the preferred frequency and mode of delivery (Slots & Ting, 2002; McLelland et al., 2009; McLelland et al., 2011; McLelland, 2019). It is not always practical to give repeated doses of medication daily; therefore, long acting antibiotics are often the medication of choice. In some European institutions, where there was no on-site veterinary support, the delivery of medication would be the responsibility of the keepers. This may have restricted the range of antibiotics available to oral medications, if keepers trained in the delivery of injectable antibiotics were not available to administer the product. Anecdotally, the delivery of oral antibiotics can be problematic, due to non-compliance, insufficient administration of the drug, and inconsistent delivery by zoo staff. Although these issues are not restricted to oral medications. This combination could lead to an overall negative outcome for macropods treated with antibiotics alone, as perhaps indicated by the high death rate (68.4%) of those treated in the European region, where as previously mentioned, treatment, by means of antibiotics alone, was more commonly used. While several pharmacokinetic studies have been reported in macropod species (Kirkwood et al., 1988; McLelland et al., 2009; McLelland et al., 2011) without adverse effects, reports of dysbiosis, particularly after the administration of oral antibiotics such as penicillin, have been reported (Gillett & Hanger, 2019). The choice of antibiotic therapy and method of delivery should therefore be made judiciously.

Kirkwood et al. (1988) recommended that macropods should be treated primarily with oxytetracyclines, an antibiotic often delivered via injection. The permanent presence of veterinary teams at Australian zoos likely assisted the administration of this medication in the 35.4% cases treated in the region, and ensured the delivery of a therapeutic drug dose. This would potentially facilitate the lower mortality rate in macropods treated with antibiotics alone in the Australian region (31.6%). Oxytetracyclines were used in only 7% of cases in the European region, and although the reason for the selection of antibiotics is unknown, the lack of veterinary personnel available to administer the medication may have influenced the selection of antibiotics other than oxytetracyclines. However, records frequently did not disclose mode of administration, therefore it is unclear how the antibiotics were administered.

Clindamycin was used to an almost equal degree across both regions, and has known antimicrobial properties in the control of anaerobic infections involving alveolar bone (Addy & Martin, 2005). In conjunction with other medications, Kane et al. (2017) reported the successful use of intravenous clindamycin to treat lumpy jaw in two red-necked wallabies. This route of administration is challenging, and the wallabies involved required long-term treatment with a range of antimicrobial products, not just clindamycin. Clindamycin has the notable benefits of being both readily available and cheap, which may explain why this has become one of the most popular drugs used in both the Australian and European institutions in our study. Although clindamycin has a favourable spectrum and is known to work well on cases involving anaerobic bacteria, including *Fusobacterium*, the oral formation is very unpalatable and requires twice daily parenteral administration; subsequently, making compliance problematic. Additionally, clindamycin is also a product widely known to have adverse side effects (Slots & Ting, 2002; Watson et al., 2017). Diarrhoea is commonly reported, which can have a negative impact on the recovery of a macropod already debilitated by lumpy jaw, potentially leading to cessation of drug use. The adverse effects of antimicrobials are well reported, and the onset of additional complications such as diarrhoea can lead to an increasingly undesirable outcome for the individual concerned (Slots & Ting, 2002; Watson et al., 2017).

Kirkwood et al. (1988) and Samuel (1983) reported that complete clinical resolution using standalone antibiotics is difficult. Results from this study support these findings. However, given the age of these papers, and developments in antibiotic usage since the 1980s, the outcome for animals treated with antibiotics alone could be expected to have improved over time. Nevertheless, as understanding of antibiotics has increased over time, antibiotic resistance has also developed and has been reported in macropods (Chen et al., 2015). Antibiotic resistance reduces the efficacy of many antibiotics used to treat conditions such as lumpy jaw (Tancawan et al., 2015; Ventola, 2015). Further investigation of microbial culture results, antibiotic selection, method of administration and relationships with health outcomes for lumpy jaw is required.

Surgery

Treatment for lumpy jaw involved surgery in 63.3% of all cases, and results from this study show that surgical intervention is more effective in treating lumpy jaw than the use of antibiotics alone. For initial cases of lumpy jaw (Australia and Europe combined), there was a significant difference in outcome by treatment type ($p = 0.02$). Treatment involving surgery resulted in fewer deaths overall (30.8%) than treatment involving antibiotics alone (50%). Macropods were significantly more likely to experience a recurrence of lumpy jaw if they received surgical intervention than if they were treated with antibiotics alone ($p = 0.006$). However, this greater likelihood of recurrence could be explained by the fact that more macropods survived surgical treatment for lumpy jaw, and consequently, more were alive to exhibit a recurrence of the disease.

The four surgical methods for addressing lumpy jaw that were identified in zoo records were the same as those previously reported in the literature. In initial surgical procedures, tooth extraction was the most common procedure carried out in both regions (Australia 42.8%; Europe 58.8%), followed by debridement (Australia 8.2%; Europe 11.8%). Other surgical treatments included the use of antibiotic impregnated beads, and root canal treatment with apicoectomy. Tooth extraction was undertaken less frequently in initial cases (combined regions 44.3%) than case recurrences (54.4%). Clarke (2003) recommended the removal of affected teeth, however tooth removal can also lead to further dental problems including malocclusion and, in the already-weakened alveolar bone, the removal of a tooth can lead to pathological fracture (Kilgallon et al., 2010) or possible recurrence of lumpy jaw. Zoo records often did not indicate if the extraction was for preventive purposes or due to associated lumpy jaw infection; therefore, the efficacy of tooth extraction as a treatment for lumpy jaw could not be determined in our study. Tooth extraction was always carried out in conjunction with topical and/or systemic antibiotics, yet without controlling for accompanying dental disease through the regular removal of plaque and calculus (Clarke, 2003; Glatt et al., 2008), these efforts may be futile (Vogelnest & Portas, 2008). In cases where surgery was undertaken, including tooth extraction, recurrence of lumpy jaw was common (43.8%).

The application of more than one surgical method was seen in nearly half of the initial cases of lumpy jaw (44.9%). Debridement was often undertaken in conjunction with tooth extraction, as recommended by Clarke (2003). The reasons behind failure of surgical treatment of lumpy jaw in the presence of necrotic bone primarily involve unresolved infection. Infection will not resolve until all sequestra have been debrided. In combination with debridement, the use of AIPMMA beads has the advantage of enabling the slow-release and direct delivery of antibiotics to the required area, without the toxic effects of some systemic antibiotics (Booth & Gage, 2008). Yet this method was only applied in 2.8% of all cases of lumpy jaw, perhaps due to few reports of the success of this therapy being carried out in macropods (Hartley & Sanderson, 2003; Vogelnest & Portas, 2008; Kane et al., 2017), or due to the complex process of manufacturing the beads. There are, however, distinct disadvantages of AIPMMA beads. Radical debridement is required prior to implantation of the beads, although this can also lead to further infection if the site is not managed during the debridement process (e.g. flushed with sterile saline) (Samuel, 1983; Hartley & Sanderson, 2003). However, once AIPMMA beads are in place, individuals may not require stressful recapture and handling, or further darting for recurrent antibiotic treatment (Hartley & Sanderson, 2003).

If asepsis is not maintained during and after surgery, surgical debridement can lead to the resulting wound becoming susceptible to further infection. Strict hygiene and aftercare are imperative for the recovery of the affected macropod, although this is often challenging when housing species that routinely live outdoors and in groups. Equally, surgery can extend the period of recovery, leading to a longer period of hospitalisation. This in turn can exacerbate stress and require the use of neuroleptic agents (Vogelnest & Portas, 2008) and anxiolytics to reduce the stress of hospitalisation. Balancing the affected macropod's need for access to its mob, and associated reduced stress, against the need to protect its physical health and preserve hygiene is challenging. This is especially the case because macropods that had a surgical procedure were found to have had a significantly longer duration of treatment than those which had antibiotics alone (74 days vs 44 days; $p = 0.001$). This pattern was observed in the Australian region (76 days vs 33 days; $p = 0.001$), but not in the European region ($p > 0.05$). This could be the result of one or more region-specific differences such as a larger sample size in the Australian region, and the more frequent permanent presence of zoo veterinarians in this region (Chapter 3, section 3.3.4), with the associated ability to facilitate surgery and provide post-surgical support onsite. However, lengthy post-surgical treatment may induce more stress, resulting in immunosuppression and reduced likelihood of recovery (Moberg, 2000); which may also help to explain the high recurrence rate observed in individuals that received surgery.

5.5.3 Treatment outcomes

The aim of treatment for lumpy jaw is to achieve a clinical resolution; however, as our study reports, this is difficult to achieve. Our research reported the most frequent outcome of treatment for lumpy jaw was the death of the macropod: over a third (34.3%) of all individuals that received treatment died as a result of the condition.

However, this figure does not give a true indication of the survivability of lumpy jaw. Often macropods are euthanased prior to the commencement of any treatment; as was the case for 18.2% of cases within this study. It is difficult to ascertain, using our available data, the extent to which this disease is treatable, as the humane euthanasia of individuals was carried out frequently; potentially to prevent further suffering and perhaps also related to other factors such as an individual's value to the collection.

The presence of veterinarians at a zoological institution may influence the rapidity and complexity of treatment for lumpy jaw, and therefore affect outcome. Post-treatment deaths were higher in European zoos than Australian zoos (50% compared to 31%). This may be related to challenges associated with the delivery of treatment to macropods housed in European institutions, in part due to the lack of permanent onsite veterinary care at some institutions. Only two of the European institutions involved in this study had access to a full time veterinary team and associated facilities (as opposed to the availability of veterinary care in all of the Australian zoos investigated). Given the higher proportion of post-treatment deaths in European compared to Australian zoos, the presence of a veterinarian may have an effect on the outcome of lumpy jaw treatment. The rapid treatment of lumpy jaw is recommended by Lewis et al. (1989), Hartley and Sanderson (2003), Jackson (2003) and Vogelnest and Portas (2008), and it is suggested that early recognition and treatment of the disease plays a major role in the expected outcome of disease. Personal observation of sampled European institutions showed that in some institutions, veterinarians visited on a weekly basis (unless required otherwise), and there was no evidence in zoo records to suggest that veterinarians routinely health checked macropods, either visually or under manual restraint. This could mean that opportunities to capture early cases of the disease were missed, and macropods were not diagnosed until the disease was clinically apparent and more difficult to treat. Equally, without the presence of onsite veterinary facilities to support the veterinarian, it is unlikely that health checks were performed under chemical restraint, or that any subsequent surgical treatment (if required) would take place. The onsite availability of a resident veterinarian would enable keepers to communicate findings of clinical signs more readily, and potentially lead to earlier diagnosis and treatment of lumpy jaw.

Recurrence of lumpy jaw is expected, and results from this study show there was widespread recurrence across regions and species. A report from the 1980s stated that disease recurrence is “relatively common” (Lewis et al., 1989, p. 396), and the present research demonstrates that recurrence is particularly commonplace. Over a third (39.6%) of treated cases of lumpy jaw experienced at least one case recurrence. There was a greater number of macropods in the Australian region that experienced case recurrence (n = 74/178 animals), than those in European zoos (n = 8/36). Some individuals (n = 6 Australian region) experienced up to three case recurrences. A high rate of recurrence was also observed, collectively across regions, in those that received surgery (43.8%). A similar result was reported when considering the Australian region alone (44.7%), whereas in the European region around a third (35.3%) experienced case recurrence post-surgery. A greater proportion of individuals received surgical intervention in the Australian region than in the European region, and subsequently, Australian macropods treated surgically, survived, with the potential to experience recurrence. Recurrence typically reflects surgical failure to remove pathogenic bacteria and necrotic tissue, and may be the result of insufficient debridement. Recurrence may also be linked to inappropriate antibiotic choices, treatment failure, changes to the oral microbiome as the result of the disease, and failure to address underlying causative factors. The ongoing environmental presence of pathogenic bacteria, but could also reflect regional survival rates. It is also possible that veterinary experience, or the time allocated to a visiting veterinarian, for example, played a role in the ability to treat macropods for lumpy jaw. Evidence from this study suggests that current surgical techniques require refinement given high failure and recurrence rates found in this study. However, irrespective of the method of treatment, 53.7% of these recurrent cases died. These figures indicate that even after treatment, macropods are likely to succumb to the disease. However, a reduction in post-surgical infections and case recurrence can be achieved by careful management of post-surgical husbandry. Of those individuals that experienced a case recurrence, 34.1% went on to achieve a clinical resolution. These data should provide confidence that in the event of recurrence, the delivery of treatment is potentially a viable and reasonable option.

Overall, 26.1% (54/207) of all macropods treated for lumpy jaw across the Australian and European regions achieved a clinical resolution. A quarter (25.1%) of all those treated in the Australian region experienced a complete resolution after treatment for an initial case. In Europe, this figure was marginally greater (27.7%), raising a question as to whether the European approach to treatment, using medical rather than surgical treatment, may have a slightly more favourable outcome. However, as discussed previously, the inconsistencies in the outcome of treatment between the regions challenges this claim. Due to confounding variables at an institutional level, including differences in surgical procedures undertaken and antibiotic products used, such a hypothesis remains speculative. Successful treatment of lumpy jaw is more likely due to the selection of appropriate treatment based on individual case presentation.

5.5.4 Risk factors

Several other variables may affect the outcome of treatment for lumpy jaw, including age, sex, and location of the oral lesions. The age at first diagnosis ranged between 0.3 and 8.9 years, and the outcome of treatment was not found to be affected by the age of the animal (p = 0.4). The sex of the animal also had no association with the overall outcome of treatment (death or resolution) (p = 0.21). However, the location of the lumpy jaw lesion had a significant effect on outcome of the condition in the Australian animals (p = 0.05); with significantly more macropods achieving a clinical resolution if lesions presented in the mandible. This result was not observed in the European macropods, although in both regions, lumpy jaw lesions were more frequently observed in the mandible.

Region

Previous research has identified a concern that the environment may influence incidence of lumpy jaw (Kido et al., 2013). Recurrence is anticipated, especially in areas where the climate is wet, as humid climates could create an environment more favourable for anaerobic bacteria to thrive in the soil (Oliphant et al., 1984). Although disease is an interaction between the host, the pathogen and the environment, favourable environmental conditions could influence the recurrence of lumpy jaw, for example following treatment macropods which are returned to the same enclosure where they initially resided, could become re-infected with pathogenic bacteria. Disease recurrence or re-infection could be reduced by the resting of enclosures, providing a substrate that reduces the presence of bacteria such as bark or sand (Witcomb et al., 2014), and by carrying out thorough disinfection and hygiene practices; for example the use of dedicated feeding trays. Previous research (Burton, 1981) has also identified the issue of bacterial contamination in soil in enclosures, and has recommended that feeding platforms should be raised off the ground to prevent faecal and bacterial contamination of feed. The findings from this present study, in addition to the findings from Chapter 3, contribute to these management suggestions, strongly recommending that facilities ensure that disinfection and hygiene management are accepted as practices of paramount importance to prevent recurrence. This is of particular importance in enclosures where lumpy jaw has been detected.

Sex

Our research identified an association between the outcome of treatment for the initial case of lumpy jaw, and the sex of the macropod. Collectively, across regions, the outcome for males was less favourable than females, as nearly a third of males (32.3%) died during treatment for the condition, compared to 22.1% of females. The same pattern was observed in both the Australian and European regions separately. It is important to note, however, that the poorer outcome for males may be based on factors other than the effects of lumpy jaw and subsequent treatment; as the uncontrolled variable of population control may have been a sex-specific factor. Zoos work towards achieving a balanced population of macropods that reflects the sex ratio observed in wild populations (Rees, 2011; Hosey et al., 2013), and excess individuals of a particular sex are often the subject of humane euthanasia for population control (Princée, 2016). For example, a male which is presenting with lumpy jaw and is surplus to demand, having little or no breeding value to the collection, may be more likely to be culled than a female in the same circumstances. We therefore advise interpreting this finding with caution, and we recommend additional research to investigate relationships between outcome of treatment and sex, while controlling for differences in euthanasia rates related to population control. In this research, the proportion of males and females for which a clinical resolution was achieved was almost the same in both regions; indicating that the outcome of treatment for lumpy jaw is not affected by sex.

Age

Several postulated risk factors for the onset of lumpy jaw have been suggested, including the presence of molar progression (Miller et al., 1978; Butler, 1981), a phenomenon affected by age as discussed in Chapter 1 (pp. 39 - 40). The age of onset of lumpy jaw in our study ranged between 0.3 years to 18.9 years, and varied between species. The age of onset in the swamp wallaby was greater in both the regions investigated in our study than the age of onset reported previously (Kido et al., 2013). Kido et al. (2013) reported the average age of onset as 3.1 (±2.1) years in this species, while results from this study indicated 5.2 (±3.25) and 5.3 (±5.59) years in the Australian and European regions respectively. In general, differences in age of onset of lumpy jaw within a species in captivity are reported to be related to institutional diet and housing differences, and other aspects of their captive environment (Butler & Burton, 1980; Burton, 1981; Wenker et al., 1999; Glatt et al., 2008; Vogelnest & Portas, 2008). However, differences between the findings from our study and those of Kido et al. (2013) may also be related to the latter having followed individuals from birth within the zoo, which gives an accurate age of onset. In contrast, in our study it was often unclear from available records whether animals may have experienced lumpy jaw at previous institutions or prior to our study period; therefore, this study's results may not indicate the true age of (first) onset, instead potentially indicating age of disease recurrence in some cases.

Although our study found no significant difference in outcome of treatment by age, our methodology had limitations. Macropods were categorised into two age groups, based on mean age of sexual maturity of macropods, across the species: but with such great variation between different species, and between the sexes, with respect to the age of sexual maturity, our results must be treated with caution. These methods were selected due the complexity of statistically analysing the 21 species involved in this study, each with differing ages of sexual maturity. The methods were streamlined for ease of analysis, although a deeper investigation into this aspect could provide more accurate findings.

Location of lesions

Lumpy jaw lesions have been reported in both the mandible and the maxilla of macropods, and have also been observed in the nasal turbinates in other animal species (Craig et al., 2016). In our research, the most frequently observed site for lumpy jaw lesions was the mandible. Our study found that macropods were 2.5 times more likely to have a clinical resolution than recurrence, if lesions were detected in the mandibular region than in the maxilla. However, we note that in nearly half of all recurrences, the lesions appeared in a new location. Kido et al. (2013) study was the first to demonstrate a left-to-right alternation in the location of lesions; this methodology was different from those in our study, which reports an alternation between the upper and lower jaw. The pattern of recurrence in the Kido et al. (2013) study may have been due to the single surgical method being employed (tooth extraction), and perhaps if additional debridement of the affected area occurred, then recurrence may not have been reported. Equally, masticatory effort will be affected with a case of lumpy jaw, even more so after surgical intervention, therefore it is reasonable to conclude that recurrence of lumpy jaw may occur in a new location, whether left, right, or involving the mandible or maxilla. Treatment selection, and the decision to treat or not, may be influenced by both the stage at which the disease has progressed, and the location of the lesion. It is harder to treat osteomyelitis and apical infections of the maxilla, due to the potential involvement of vital anatomical structures in the maxilla; this makes surgery difficult in this area. Maxillary cases of lumpy jaw might be euthanased without treatment due to the difficulty of treating lesions in this area. It is easier to get clean margins around infected bone in the mandible, which might lead to better outcomes for mandibular cases of lumpy jaw. It would have been beneficial to determine which teeth are more likely to be affected by lumpy jaw lesions, with molar teeth previously reported to be more likely to be associated with lumpy jaw (Miller et al., 1978; Butler & Burton, 1980). This information would greatly assist in the clarification of whether molar progression is a risk factor for lumpy jaw, as previously suggested (Finnie, 1976; Miller et al., 1978; Burton, 1981; Bird et al., 2002; Clarke, 2003; Lentle et al., 2003; Bakal-Weiss et al., 2010; Antiabong et al., 2013a). In addition, as molar progression is correlated with age (Jackson, 2003), future studies could aid in the development of an algorithm by which veterinarians could predict and anticipate the age at which lumpy jaw is likely to occur. This could facilitate a program of increased observations of individuals of particular ages or stage of development.

Species

Some species and genera are more susceptible to lumpy jaw (see results in Chapter 3, pp. 71 - 72; Chapter 4, p. 121); however, the true outcome of treatment in relation to species is difficult to evaluate from our data, due to the small number of individuals from most species that were available for inclusion in the study. The more popular species (the red kangaroo and the red-necked wallaby) are represented by large numbers in collections, with corresponding large sample sizes in our study. The outcome-of-treatment analyses showed that both the red kangaroo and red-necked wallabies have a high case recurrence for those treated with surgery in the Australian region (51.4% and 52.4% respectively). In contrast, the red-necked wallabies housed in European institutions experienced fewer case recurrences (20%) after surgery than their Australian housed conspecifics. However, 66.7% of European housed red kangaroos experienced case recurrence post-surgery; although these figures are based on a small sample size of red kangaroos which received surgery in the European region (n = 3), thereby confounding results. The small number of red kangaroos treated with antibiotics in the European region exhibited an equal number of deaths and clinical resolutions (50% respectively). This could be the result of the selection of specific antibiotics, but without a detailed review of the specific cases, this can only be speculated. Red-necked wallabies in Europe received treatment for lumpy jaw using both antibiotic and surgical approaches, in almost equal numbers. Results for those which died post-treatment indicate that this species does not respond as well to antibiotics alone, compared to treatment involving surgery. There was a greater number of deaths in those that received antibiotic therapy alone (87.5%) compared to those that received surgery (40%). Red-necked wallabies treated for lumpy jaw in the Australian region showed similar results, with 100% (n = 1) death rate for treatment using antibiotics alone compared to 33.3% (n = 7) in those treated with surgery. Similar to the European region, the figures for Australian red-necked wallabies are also based on very small sample sizes, therefore it would be speculative to make an assumption as to the efficacy of treatment approach in this species.

The red-necked wallaby has been the subject of several investigations into the treatment of lumpy jaw (Hartley & Sanderson, 2003; Bakal-Weiss et al., 2010), and some success has been reported. The use of AIPMMA beads provided a clinical resolution in one case of lumpy jaw in a red-necked wallaby (Hartley & Sanderson, 2003). However, this individual died a year after treatment, of unrelated causes. Given the results from our study, it could be argued that this red-necked wallaby did not survive long enough to determine if recurrence would occur. The mean period of quiescence, as determined in our study, identified that recurrence frequently occurs 1.2 years after a case resolution. Therefore, the Hartley and Sanderson (2003) case study may not represent a true success, because the individual did not live long enough to see if a recurrence would occur. In comparison, another treatment method was successfully trialled by Bakal-Weiss et al. (2010). Chlorhexidine varnish was used to fill a lumpy jaw lesion in a red-necked wallaby. As recurrence had still not occurred 2.2 years following treatment, this method could more confidently be considered a success in this individual. Results from this present study show that treatment for lumpy jaw, specifically in the red-necked wallaby, is not successful, specifically in those receiving antibiotics alone. This could indicate that this species is more likely to succumb to the effects of the disease and its treatment, than previous studies suggest (Hartley & Sanderson, 2003; Bakal-Weiss et al., 2010), however species-specific studies would be needed to test this suggestion. Alternatively, the stress of treatment in this often-fractious species may complicate treatment. Therefore, treatment using antibiotics alone in the red-necked wallaby is not recommended (Kirkwood et al., 1988).

Species type has been reported previously to be a factor in outcome of treatment for lumpy jaw (Kido et al., 2013). Treatment involving the extraction of teeth with supportive antibiotic therapy was shown to be more effective than antibiotics alone in the swamp wallaby in a Japanese institution (Kido et al., 2013). Results from the Australian region support the findings from the Kido et al. (2013) study of a high recurrence rate in this species. However, in the Australian region, swamp wallabies were only treated using surgical intervention; therefore, no comparison can be made with regard to the outcome of treatment using antibiotics alone. In the European zoo records, swamp wallabies (n = 2) did not receive treatment for lumpy jaw. One individual died and one was reported to recover, although given the known pathology of lumpy jaw, recovery without treatment would be expected to be unlikely, and we cannot rule out the possibility that this animal was either incorrectly diagnosed, or that medical treatment was delivered and was either not recorded or was missing from the records.

Outcome of treatment for lumpy jaw was observed to vary between species in this study. Yellow-footed rock wallabies treated for lumpy jaw with a surgical procedure in Australia had a high rate of case resolution. This may reflect institutional practices in the early detection of lumpy jaw in this particular species. On an institutional level, this species was housed in large numbers, and records indicated that tri-monthly checks, specifically aimed at detecting early signs of lumpy jaw, were undertaken in one of the four institutions sampled. The results from this study indicate that frequent oral health checks provided a positive outcome for this species, with 25% of those treated with surgery achieving case resolution. The effectiveness of early surgical intervention was not measured in this study, however the results suggest that perhaps early detection and a tri-monthly protocol could be implemented in other institutions and species to improve lumpy jaw outcomes. Information regarding species susceptibility and likely outcome of treatment may influence the species that zoos choose to house in the future. Equally, species-specific survival curves for lumpy jaw would demonstrate which species are most appropriate to maintain in captive collections to minimise occurrence of this disease.

5.5.5 Duration of treatment

Combined data across both geographic regions showed the mean duration of treatment for lumpy jaw was significantly longer in recurrent cases than the initial case (p = 0.03). A similar pattern was found in both the Australian and European regions separately, significantly so in the European region (p = 0.05). This may imply that greater treatment effort was given to individuals that experienced recurrence, or that the issue was more persistent in recurrent cases. The method of treatment in Europe favoured antibiotics alone, yet results did not reflect that the method of choice in the European region had an influence on the duration of treatment (p > 0.05). Results collectively analysed for both regions demonstrated that the use of antibiotics alone led to a significantly shorter duration of treatment than the duration for individuals treated with surgical intervention (p = 0.001), with Australian institutions also reflecting this trend (p = 0.001). However, this observation could be related to the large percentage of macropods that died during treatment involving antibiotics alone (50%). Interestingly, this finding was reversed in the European zoos studied. Individuals that received surgical treatment halved the treatment duration compared to those receiving antibiotics alone (antibiotics - 61 days; surgery - 32 days). Possible reasons for this difference are discussed below.

Several potential factors may affect the delivery and subsequent duration of treatment. The financial constraints of treatment affect all animal collections in institutions, but equally the presence of an on-site veterinarian will affect treatment options. In half of the European collections visited, veterinary presence and facilities were minimal; affecting the level and type of treatment that could be delivered, which may have contributed to the higher number of macropods that were treated with antibiotics alone. Equally, the records in these institutions were often incomplete, and did not reflect ongoing investigations and treatments, thus potentially affecting the results for the European region.

The duration of treatment and treatment effort may also be related to the breeding or conservation value of the individual undergoing treatment. This includes both its value to the collection and to the conservation of the species. The species housed across the institutions varied, although two species treated for lumpy jaw in the Australian region are reported as ‘Endangered’ on the IUCN Red List; these included the Goodfellow’s (*Dendrolagus goodfellowi*) (Leary et al., 2016) and Matschie’s tree kangaroos (*D. matschiei*) (Ziembicki & Porolak, 2016). In contrast, generally species housed in Europe were not endangered.

5.5.6 Management recommendations

Based on the results of this research, the use of both antibiotics and surgical intervention is recommended, but should be considered on a case-by-case basis. The selection of an antibiotic should be based on the results of biopsy of affected tissue and/or bone with microbial culture and sensitivity being performed. Further research into the efficacy of specific antibiotics, including pharmacokinetic trials in common species of macropods, is strongly recommended. Ideally, a longitudinal study in captive macropods following outcomes of specific surgical techniques and case recurrence is warranted and much needed.

Lumpy jaw is difficult to treat, but early diagnosis, along with treatment using systemic antibiotic therapies and surgical intervention, may improve the outcome for macropods (Fagan et al., 2005). Routine health checks under anaesthesia should always involve a thorough examination of the oral cavity (Wiggs & Lobprise, 1994); and any non-routine examination or procedure should take advantage of having the individual immobilised, and a full dental examination including radiography should be undertaken if indicated.

Lumpy jaw is not only difficult to treat, it is difficult to detect during the primary stages of development. Therefore, tri-monthly screening for the disease could assist with early diagnosis and the provision of a suitable and effective treatment regimen. However, the risks associated with the capture and subsequent anaesthesia required to perform a clinical examination need to be balanced against the benefits of screening. Reliance on keeper observation and being alert to subtle changes in behaviour, for instance, difficulty with mastication, may be critical for the early detection of lumpy jaw in captive macropods. However, keeping staff should also be advised of the more likely occurrence of lesions within the mandibular region, and well-trained in the early detection of both clinical and behavioural signs of lumpy jaw. In addition, keepers should be encouraged and enabled to undertake conditional training for medical examination of the jaw area, to habituate the macropods to be amenable to regular inspection of the head and neck. Behavioural training alone could not replace veterinary examinations under anaesthetics, however, it would greatly assist the veterinary team, and the welfare of macropods in the institution's collection, by reducing the frequency of oral cavity examinations under general anaesthesia, with its associated risks and costs.

Recurrence of lumpy jaw occurs most frequently around > 12 months post initial resolution. Therefore, observations of individuals that have been previously affected should be diarised for a re-check of the oral cavity around this time. Particular care should be taken to avoid looking solely for recurrence in the same location as previous lesions; as recurrence can, and often does, occur in a new location. Once clinical signs are observed, macropods with active lumpy jaw lesions are likely to be shedding pathogenic bacteria into the environment. The immediate removal and isolation of the affected macropod is recommended, for the duration of treatment, to prevent recurrence. In addition, enclosures should be thoroughly cleaned and disinfected, where possible.

This research reported that treatment for lumpy jaw is influenced by lesion location. Treatment is more likely to reach a clinical resolution if lesions are in the mandible. The treatment of cases with mandibular lesions should therefore be encouraged. However, recurrence is likely in cases with maxillary lesions. Therefore, it is recommended that consideration should be given to the welfare of the individual, as well as the value of animal to the collection, before embarking on potentially unrewarding, lengthy and painful treatments, especially in individuals presenting with lesions in the maxillary region. Investigations into preventative measures, including analysis of current housing, husbandry and hygiene, are highly recommended.

Post-surgical management of macropods, by controlling environmental bacterial, will reduce the risk of further infection or recurrence. The oral cavity is abundant with bacteria and is difficult to keep clean. Therefore, avoiding substances in the diet that may adhere to the wound or lead to further abrasion, such as pellets and sharp hay, may be advisable following oral surgery. Food material may be impacted into the tooth socket during mastication (Fagan et al., 2005), which may lead to bacterial presence and subsequent recurrence of disease.

5.5.7 Limitations of this research

Retrospective studies using veterinary and other records, although usually straightforward to access, present challenges, particularly with respect to the reliability of the data being collected. Records available for this study were often incomplete or illegible (paper records), and some had to be translated from another language. Interpretation of translated records may have been lost in translation; although mechanisms were in place to prevent this from occurring, by checking the translation with bilingual veterinarians.

Lumpy jaw is syndromic and has several clinical signs requiring clinical interpretation to reach a diagnosis. Cases of lumpy jaw, identified from medical records, were based upon the clinical description given, and therefore relied on the records accurately reporting on the presence of true lumpy jaw. However, records may not have always been accurate in this regard; for example, the clinical presentation could be confused with a grass seed abscess, or with an oral foreign body. The definition of lumpy jaw, developed for and used in this study, may not have been robust enough to detect the difference between true lumpy jaw and these other issues, resulting in false positives. In addition, cases of lumpy jaw reported in zoo records were subject to the interpretation of clinical signs by the diagnosing clinician. Some clinicians do not diagnose lumpy jaw until proliferative bony change is present; while for other clinicians, a soft tissue abscess may be reported as lumpy jaw (W. Boardman, personal communication, 17th August 2016). This potential issue became apparent early in the study and resulted in the development of the case definition, as there was much discussion between veterinary professionals as to what constitutes a case of lumpy jaw. This confusion could be avoided if a prospective study of case diagnoses was undertaken, using clear guidelines for the diagnosing veterinarian to follow to detect cases. This would include clinical presentation and prognostic categories for lumpy jaw.

The study population for this research comprised of macropods that received surgical and/or medical treatment for lumpy jaw. Animals diagnosed with lumpy jaw are often euthanased, or experience an unassisted death, before any treatment can commence. Euthanasia is often reported as a 'treatment' for lumpy jaw, however, in this study individuals that were euthanased, or experienced an unassisted death, were removed from the study population, if they did not receive any other surgical or medicinal treatment. Thus, the study population was defined by two treatments options, rather than three; which had an effect on the overall population numbers. Although removal of individuals on the basis of euthanasia as an outcome (in the absence of treatment) is justified, a larger dataset would provide more robust results.

Large sample sizes provide strength to scientific research, however they are not always possible to achieve. Zoo-based research inevitably involves working with a small number of subjects, and to minimise this, data were collected from several institutions. Nevertheless, undertaking research in a smaller number of zoos would have enabled more detailed analysis in areas of clinical interest, including findings from microbial culture, and the use of vaccinations and oral varnishes. This may, however, have resulted in very small cohorts which would further impact upon the results, especially in areas such as the factor analysis, where small numbers were already present.

Sample size has an influence on the ability to utilise specific statistical tests, and consequently this has an effect on the robustness of the results produced. Although the total number of macropods involved in this study overall was large, the figure is not necessarily indicative of the captive population. Zoo numbers are potentially small due to the practicality of housing, and the number of individuals treated was often small. Figures for the European region were particularly small and this would have impacted on the statistical strength of the results. In addition, macropods frequently had secondary infections; veterinary records indicated there were often accompanying respiratory infections. Therefore, the antibiotics reported in this study may not have been prescribed on the grounds of the presence of lumpy jaw lesions, but perhaps to assist with the treatment of additional systemic infections.

5.5.8 Future research

There are several surgical and medical approaches available to treat lumpy jaw. However, as this study has demonstrated, the outcome of treatment often results in the death of the individual or recurrence of the disease. There would be distinct welfare advantages to finding a treatment regimen that is not only effective, but also less invasive than currently used methods. As a result of this study, it is suggested that further research should be conducted into non-invasive treatment methods, similar to the use of chlorhexidine varnish as performed by Bakal-Weiss et al. (2010). The pharmacologic concept behind the use of sustained-release chlorhexidine varnish involves the product's ability to prolong the exposure of the drug in the oral cavity, thus improving its action on the lesion site. Although it is challenging to administer chlorhexidine to caudal teeth, it would be beneficial to investigate this treatment option further, given the potential advantages of its use, including the reduced need for repeated handling, and its status as a minimally invasive procedure, thereby reducing the risk of secondary infections. This research identified a very small number of cases in which this product was used in conjunction with antibiotic and surgical therapies. However, the capacity to investigate this treatment modality further was restricted given its infrequent use, as reported in zoo records. Equally, other treatment approaches could be investigated, including the use of AIPMMA beads, and endodontic therapies. Some authors have claimed success with these treatments (Hartley & Sanderson, 2003; Fagan et al., 2005), however due to the low sample sizes in those studies, their efficacy and success is not yet established.

Evidence suggests that treatment for 'lumpy jaw' and other bone infections has been successful in cattle and horses using intravenous sodium iodide or oral potassium iodide (Walker & McKinnon, 2002; P. Ryder-Davies, personal communication, 10th

August 2017). Although there are known side effects from using these treatments, for instance, ulcers at the site of administration, there are aspects of these treatments that could be advantageous in the zoo industry; if the disease is caught in the early stages, then this option may prevent the need for invasive, expensive and often unrewarding surgery. However, the efficacy of these treatments in macropods is also yet to be established.

A longitudinal prospective investigation of the efficacy of a vaccine to prevent lumpy jaw is advocated. A vaccine would be quick to administer, and could also reduce the need for the surgical or therapeutic intervention typically required to treat this disease. Furthermore, the efficacy of antibiotics used in the treatment of lumpy jaw is continually undermined by the presence of resistant strains that appear insensitive to antibiotic therapies currently used. An investigation into bacterial species isolated and antibiotic susceptibility could assist clinicians with drug selection most appropriate for the specific microorganism(s) cultured. Ultimately, the results of such studies could have an influence on the outcome of disease and would greatly assist those using antibiotics to treat lumpy jaw. Blind clinical trials into the efficacy of both vaccinations and antibiotics would be beneficial to clinicians and zoo staff working towards preventing and treating lumpy jaw in their kangaroo mobs.

5.6 Conclusion

The treatment of lumpy jaw in captive macropods remains a challenge for veterinarians. Overall, a greater number of cases of lumpy jaw in this study received surgical intervention than antibiotics alone, although a regional preference was observed for the use of antibiotics alone in the European region. The duration of treatment was significantly longer in cases involving surgery, and in recurrent cases. Recurrence can be expected; especially if initial lesions present in the mandible.

Recommendations include behavioural training by zoo staff to facilitate oral examination, and post-diagnosis enclosure sanitation. Research should be encouraged into preventative and non-invasive measures, such as vaccination and long-acting medicaments, including sustained-release varnishes. There are several additional potential factors that may affect the outcome of treatment for lumpy jaw, however these will require investigation on an institutional level.

Rather than providing a complete knowledge of the most efficacious treatment for lumpy jaw, the results from this study have generated several hypotheses requiring further investigation. Fundamentally, they convey that treatment for lumpy jaw should be approached on a case-by-case basis, rather than expecting a particular treatment to be the most effective option across all individuals and circumstances. Despite best efforts being made to treat this refractory disease, mortality rates remain high; prevention therefore, may be better than cure.

Chapter 6

Use of computed tomography (CT) to report prevalence of lumpy jaw in wild western grey kangaroos (*Macropus fuliginosus*)

6.1 Introduction

Lumpy jaw is commonly observed in captive macropods, with reportedly high prevalence, morbidity and mortality (Jackson, 2003). In wild macropods, this disease is considered rare (Wallach, 1971; Vogelnest & Portas, 2008). However, its prevalence in the wild is largely unknown, with few studies reporting the presence, absence or extent of lumpy jaw in wild populations (Arundel et al., 1977; Miller & Beighton, 1979; Vogelnest & Portas, 2008; Borland et al., 2012). Previous studies have been largely opportunistic, with lumpy jaw detected during the course of another project such as research on mortality in eastern grey kangaroos undertaken by Borland (2006) and Borland et al. (2012). A systematic study of lumpy jaw in wild macropods would provide an indication of the endemic presence of this disease in wild populations. This information could then be used to assist those managing disease in captive populations, by highlighting potential risk factors for disease development. Here, we report a study investigating the prevalence of lumpy jaw in wild western grey kangaroos in the metropolitan region of Perth, Western Australia.

6.1.1 Risk factors

Lumpy jaw in wild kangaroos has been reported in a number of Pleistocene fossils of mandibles from giant kangaroos (*Macropus titan*), estimated to be 26,000 years old (Horton & Samuel, 1978). Early studies of lumpy jaw in wild macropods by Tomlinson and Gooding (1954) provided no suggestion as to a cause for this disease, however Borland et al. (2012) suggested that the disease in wild individuals was associated with periods of extreme environmental stress, such as drought. In their study, Borland et al. (2012) reported that limited access to essential resources and a decrease in grazing material led to overcrowding, and subsequent faecal contamination of pasture land. There is a general acceptance that environmental contamination with infected faeces or discharge is a significant factor in the development of lumpy jaw (Fox, 1923; Beveridge, 1934; Burton, 1981; Vogelnest & Portas, 2008). Bacterial transmission during grazing via faecal contamination on the ground is likely in these situations, and may also occur through unintentional or intentional coprophagic behaviour (Bennett et al., 2009). The combination of stress-induced immunosuppression and exposure and ingestion of pathogenic faecal bacteria, is reported to increase disease risk (Vogelnest & Portas, 2008; Borland et al., 2012).

6.1.2 Species affected

Lumpy jaw was originally thought to be a disease of captivity (Samuel, 1983); however, more recent reports have described the disease in wild macropod species across Australia (Borland et al., 2012). Lumpy jaw is reported to affect all species of macropod (Jackson, 2003), and has been detected in several wild species, including swamp wallabies, tammar wallabies (Arundel et al., 1977), eastern grey kangaroos (Borland et al., 2012), red kangaroos (Tomlinson & Gooding, 1954) and red-necked wallabies (Kirkpatrick in Horton and Samuel (1978), p. 280; Munday, 1978). Calaby and Poole (1971) stated that lumpy jaw in wild kangaroos was common, and also suggested species-specific susceptibilities to the disease, with the red-necked wallaby being more susceptible than the red kangaroo, followed by the grey kangaroo. Borland et al. (2012) found a high prevalence of lumpy jaw (54%) in the eastern grey kangaroo. These findings differ not only from those of Calaby and Poole (1971) but also from several other studies which have reported the prevalence of lumpy jaw to be rare in wild kangaroos (Wallach, 1971; Butler, 1981; Vogelnest & Portas, 2008). Disease in captive macropod species has in some measure, been more thoroughly investigated than in wild populations. This is likely due to the challenges involved in detection and diagnosis of disease in wildlife populations.

6.1.3 Disease detection

The detection and diagnosis of disease in wild animals is challenging, as normal health parameters are often unknown and clinical examination without chemical immobilisation is often not possible (Wobester, 2006). Disease detection typically occurs following mass die-offs, major observed behavioural changes, clinically observed health abnormalities, or direct investigation of a particular species (Wobester, 2006; Stallknecht, 2007). In free-ranging populations, lumpy jaw is often undetected until the disease has reached an advanced stage, when death commonly ensues due to an inability to masticate (Vogelnest & Portas, 2008). Diagnosis of lumpy jaw in wild macropods may occur as result of direct observation of animals at close range (Tomlinson & Gooding, 1954), or as Borland et al. (2012) reported, during investigations of dead animals. Whilst investigating mortalities in wild eastern grey kangaroos, Borland et al. (2012) reported lumpy jaw lesions in the skulls of animals found dead at Serendip Wildlife Sanctuary, Victoria. Diagnosis was based on visual observation of osteolytic changes; which in live animals may only be visible through the use of radiographic tools. In live animals, diagnosis of lumpy jaw is typically undertaken through physical examination of the oral cavity and bacterial culture; in addition, radiographs may be carried out to confirm bony involvement (Barrie, 2003; Jackson, 2003; Vogelnest & Portas, 2008). Computed tomography (CT) has also been performed to diagnose lumpy jaw in captive kangaroos (Melbourne Zoo, 2007; Lee et al., 2011), however its use in wild specimens to date has been limited (Lee et al., 2011).

Computed tomography is a non-invasive diagnostic tool used to create detailed 3D images of bone and soft tissue (Littleton & Durizch Littleton, 1996). To study lumpy jaw in wild kangaroos using CT, one option is to use carcasses sourced from a population management cull; which would provide a cross-sectional sample population for detecting the prevalence of lumpy jaw in wild kangaroos. The western grey kangaroo has been the subject of several such population management culls in recent years, with its population having been estimated to exceed 5.25 million (Burbidge et al., 2016). Carcases from these culls have provided an opportunity to report on the prevalence of lumpy jaw in this common macropod species, under normal environmental conditions, as opposed to the drought conditions present during the Borland et al. (2012) study. These data may provide an indication of the endemic status of lumpy jaw disease in free-ranging populations; information which may be utilised by macropod managers and carers to maintain health and reduce the risks of lumpy jaw in captivity.

6.2 Aims

Given that lumpy jaw is considered rare in wild populations (Wallach, 1971; Vogelnest & Portas, 2008), we hypothesise that prevalence of this disease in the sample population will be lower than the prevalence reported in captive animals of the same species (2.6%) (Vogelnest & Portas, 2008). This is the first systematic study of lumpy jaw in wild populations of western grey kangaroos using CT as a diagnostic tool. This study will investigate the prevalence of lumpy jaw in two, non-confluent, isolated wild populations of western grey kangaroos by utilising kangaroos culled as part of a population management program; reporting on disease presence or absence and potential environmental risk factors for the disease.

6.3 Methods

6.3.1 Sample collection and preparation

Study sites

Kangaroo skulls were sourced following a population management program at two sites, Thomson's Lake Nature Reserve and Melville Glades Golf Club (hereafter referred to as Thomson's Lake and Melville Glades respectively). Thomson's Lake encompasses a 550 hectare site situated 22 kilometres south of Perth city centre, on the Swan Coastal Plain of Western Australia. Thomson's Lake covers an area of 150 hectares when full and is surrounded by a belt of rushes (*Baumea articulata* and *Typha orientalis*) and couch grass (*Cynodon dactylon*). The vegetation advances as the lake recedes during the hot, dry months of summer and autumn, and these perennial grasses and sedges supplement the lower storeys of the surrounding woodlands to provide resident western grey kangaroos with year-round feed. The reserve is surrounded by a well-maintained 2.4 metre high chain-link fence topped with a barbed wire outrigger and an electric wire. The fence extends into the ground to contain the kangaroos. When the reserve was first fenced, a 1080 (sodium fluoroacetate) baiting program was undertaken to remove potential predators. The resident kangaroo population subsequently increased, and by 2005 the numbers were considered to be a problem to native flora through over-grazing and facilitation of the spread of introduced weeds, as well as a threat to water bird nest sites. There was a resident population of 1100 western grey kangaroos in 2006, when the population control program was undertaken.

The second site was Melville Glades, a site of approximately 67 hectares located in the suburb of Leeming, 14km south of Perth city centre. The golf course itself consists of perennial grasses that are sprinkler-irrigated, fertilised and mowed throughout the year. This results in continual growth of nutritious grasses. Natural vegetation occurs between the fairways and consists of annual grasses and forbs, active from autumn to spring. At the end of 2007, the resident population of kangaroos at this reserve was approximately 65. Although the golf course was surrounded by a 1.8 m high fence, the fence line was breached in several places, and the entrance was permanently open, enabling the free movement of the kangaroos.

Sample collection

Western grey kangaroo skulls used in this study (from the aforementioned sites) were sourced and prepared for a previous study (Mayberry, 2009; Mayberry et al., 2018), and were originally part of a management cull endorsed by the Conservation Commission of Western Australia 2005. The cull was overseen by representatives from the then Department of Environment and Conservation (DEC) and the Department of Local Government and Regional Development, who were responsible for the administration of animal welfare legislation, including ensuring humane destruction of the animals. In accordance with the national Code of Practice for the Humane Shooting of Kangaroos (Department of the Environment and Heritage 1990), a high-powered rifle shot to the head was used to kill adult and sub-adult kangaroos. All shooting was undertaken by professional shooters with official licences (DEC Licence TF003341). All female kangaroos culled were examined for the presence of pouch young; all pouch young were killed immediately by decapitation, heavy blow to the skull, or shooting, based on the size of the pouch young and in accordance with the Code of Practice (Department of the Environment and Heritage 1990).

Between May 2006 and March 2007, the DEC culled 1032 adult and sub-adult kangaroos from the Thomson's Lake Reserve to reduce the impact of the kangaroos on the native vegetation and biodiversity. A total of 292 male and 244 female kangaroos, of mixed ages, were culled from the Reserve between May and July 2006, and a further 496 kangaroos of mixed age and sex between August 2006 and February 2007. A management cull at the Melville Glades Golf Course took place in September 2007.

The sex of each individual kangaroo was recorded at the time of culling. Age was estimated by molar progression (Kirkpatrick, 1964; 1965; McLeod et al., 2006) and by comparing the head length with animals aged by molar progression (Poole et al., 1982).

For the purposes of this study, a sample of skull specimens was taken from these culled populations for diagnostic imaging.

Preparation for diagnostic imaging

Initially, visual examination of the skulls was performed to select suitable (whole or near whole) skulls for CT examination. Fragmented specimens were reconstructed and secured using a Bosch® (Robert Bosch GmbH, Clayton South, Victoria) Glue Pen (3.6 V Lithium-ion all-purpose glue pen). The specimens were placed in a partitioned cardboard box, capable of securely holding six skulls, for CT scanning in these batches (Figure 6.1).

Figure 6.1: Western grey kangaroo skulls partitioned in preparation for CT scanning.

6.3.2 Diagnostic methods

Specimens were considered as 'Complete' (all parts of the mandible were present but might have been fragmented), or 'Incomplete' (one hemi-mandible or large sections of the mandible were missing). All bones of each skull that were available were evaluated.

Visual observation of skulls

Following Miller and Beighton (1979) and Borland et al. (2012), initial observation of the skulls was performed to identify signs of bony proliferation in the mandible and/or maxilla. Using diagnostic criteria similar to those used by Borland et al. (2012), we categorised kangaroo skulls based on visually observed bone loss. Each kangaroo skull was placed into one of four categories, from normal bone through to osteoproliferative change, with the researchers considering all samples with any osteolytic changes to be cases of lumpy jaw (Table 6.1). However, adopting the approach taken by Miller and Beighton (1979) we modified the criteria to only include lesions that appeared proliferative, in order to reduce the risk of over-representing the prevalence of disease.

Table 6.1: Classification of osteoproliferative and osteolytic changes in relation to lumpy jaw adapted from Borland et al. (2012).

Category	**Skull characteristics**
Normal	No osteolytic change.
Minor	Osteolytic bone lesion(s) present but no visible alteration of the physical dimensions was observed.
Major	Significant osteolytic bone lesion(s) were observed.
Osteoproliferative	Proliferative bony lesion(s) where observed visibly altering the dimensions of the jaw bone(s).

6.3.3 Diagnostic imaging – computed tomography

CT was used to support the findings from visual observations and to confirm bony proliferation and osteolytic change. All specimens were scanned using a Siemens® 16 slice SOMATOM Emotion Scanner (Siemens Healthcare Pty Ltd, Bayswater, Victoria).

Exposure Factors: 130 kV 250 mAs. Clinical review of the CT images, including the control specimen, was carried out by a Recognised Specialist in Veterinary Radiology.

Helical scans were performed at a slice thickness of 0.75 mm through the specimens using a 16 x 0.6 mm detector array and a 0.75 mm slice thickness. The specimens were scanned rostral-caudal at a pitch of 0.55. The scan field was set to include all relevant anatomy.

Images were reconstructed using 'syngo' CT Workplace software (Siemens, Erlangen, Germany). The images were reconstructed in the transverse plane using an Extremity algorithm and U90s Ultra Sharp reconstruction kernel (Seimens Medical, 2007). Additional series were reconstructed using a Soft Tissue algorithm and B50s Standard reconstruction kernel.

Multiplanar Reformats (MPR) were created in sagittal and dorsal planes (Figure 6.2 a, b) from the initial Extremity U90s Ultra Sharp reconstructions. 3D surface renderings were created from the Soft Tissue B50s reconstructed series (Figure 6.3).

A skull specimen from a wild red kangaroo of unknown age and sex, confirmed to have lumpy jaw, was the case control for this study.

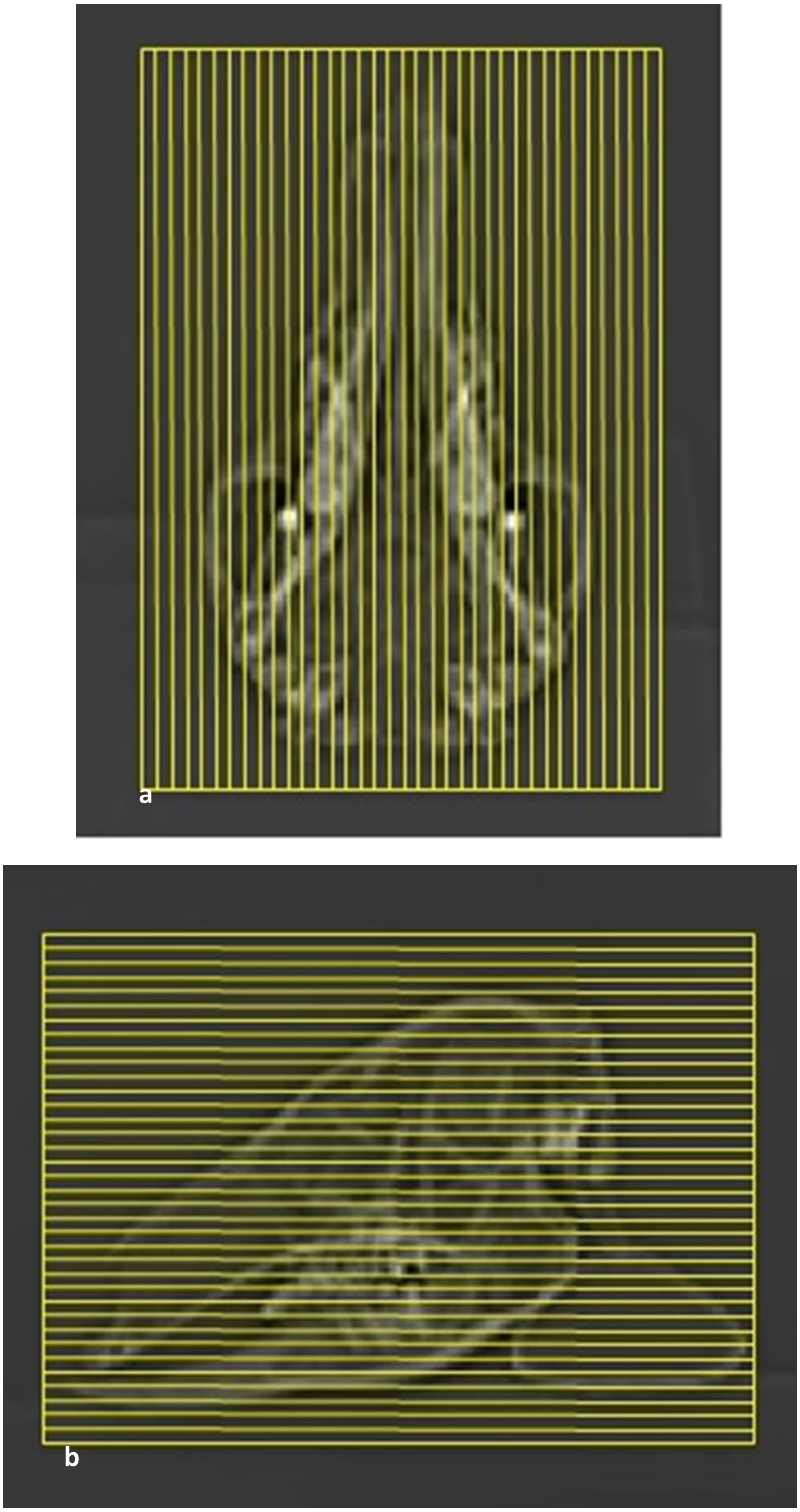

Figure 6.2: Multiplanar Reconstruction (MPR direction shown in yellow lines) of western grey kangaroo skulls in a) sagittal and b) transverse slices.

Figure 6.3: 3D Surface rendering of western grey kangaroo skulls.

6.3.4 Statistical analysis

Power analysis was undertaken to determine population requirements for the study, using Epitools (Sergeant, 2016). True prevalence was assumed at 0.02, Se 0.95, Sp 0.95, confidence level 95%. The calculated required sample size was 121. Microsoft® Excel 2016 was used to calculate descriptive statistics and prevalence of lumpy jaw for each population of western grey kangaroos, sex ratios, and the mean age of animals from each location (±SD).

6.4 Results

6.4.1 Population

The population comprised of 335 western grey kangaroos, of which 127 specimens were considered suitable for visual and CT analysis; 121 from Thomson's Lake Reserve and six from Melville Glades Golf Course.

A total of 103 (81.1%) of the specimens presented for CT were complete, and 24 (18.9%) were incomplete, having one hemi-mandible or large section of the mandible missing. In addition, it was noted by the reviewer that five individuals had missing rostral teeth, and one had a section of caudal temporomandibular joint (TMJ) missing.

Sex

Sex was determined for all 127 individuals using records from the culls, and included 75 (59.1%) males and 52 (40.9%) females (Table 6.2).

Age

Age was determined for 125 (98.4%) specimens using records from the culls. The mean age (±SD) for the population was 4.5 years (±2.61) and ages ranged between 1.0 – 13.6 years (male: *M* = 4.4, SD = 2.17, range: 1.1 – 9.9 years; female: *M* = 4.7, SD = 3.15, range: 1.0 – 13.6 years) (Table 6.2).

6.4.2 Prevalence

The visual observations and CT scans of the 127 western grey kangaroos from Thomson's Lake Reserve and Melville Glades Golf Course did not detect lumpy jaw in any specimens. The detected prevalence of lumpy jaw in the overall population of western grey kangaroos from the two locations was 0% (95% CI: 0.0 – 2.9) (Table 6.2).

Table 6.2: Specimen data and prevalence of lumpy jaw in a population of isolated wild western grey kangaroos from two locations: Thomson's Lake Reserve and Melville Glades Golf Course, WA.

Source (n)	**Sex ratio m:f**	**Mean age (±SD)**	**Age range Years**	**Lumpy jaw**	**Prevalence (95% CI)**
Thomson's Lake (121)	71:50	4.5 (2.61)	1.0 – 13.6	0	0% (0.0 – 45.9)
Melville Glades (6)	4:2	5.8 (2.38)	2.5 – 8.8	0	0% (0.0 – 3.0)
Total (127)	75:52	4.5 (2.61)	1.0 – 13.6	0	0% (0.0 – 2.9)

The CT study of the red kangaroo with lumpy jaw lesions (control), confirmed bilateral lesions in the mandible, with the right mandible more severely affected than the left. Findings from the CT study of the red kangaroo skull are presented in Figure 6.4 a, b, with a full report in Appendix F.

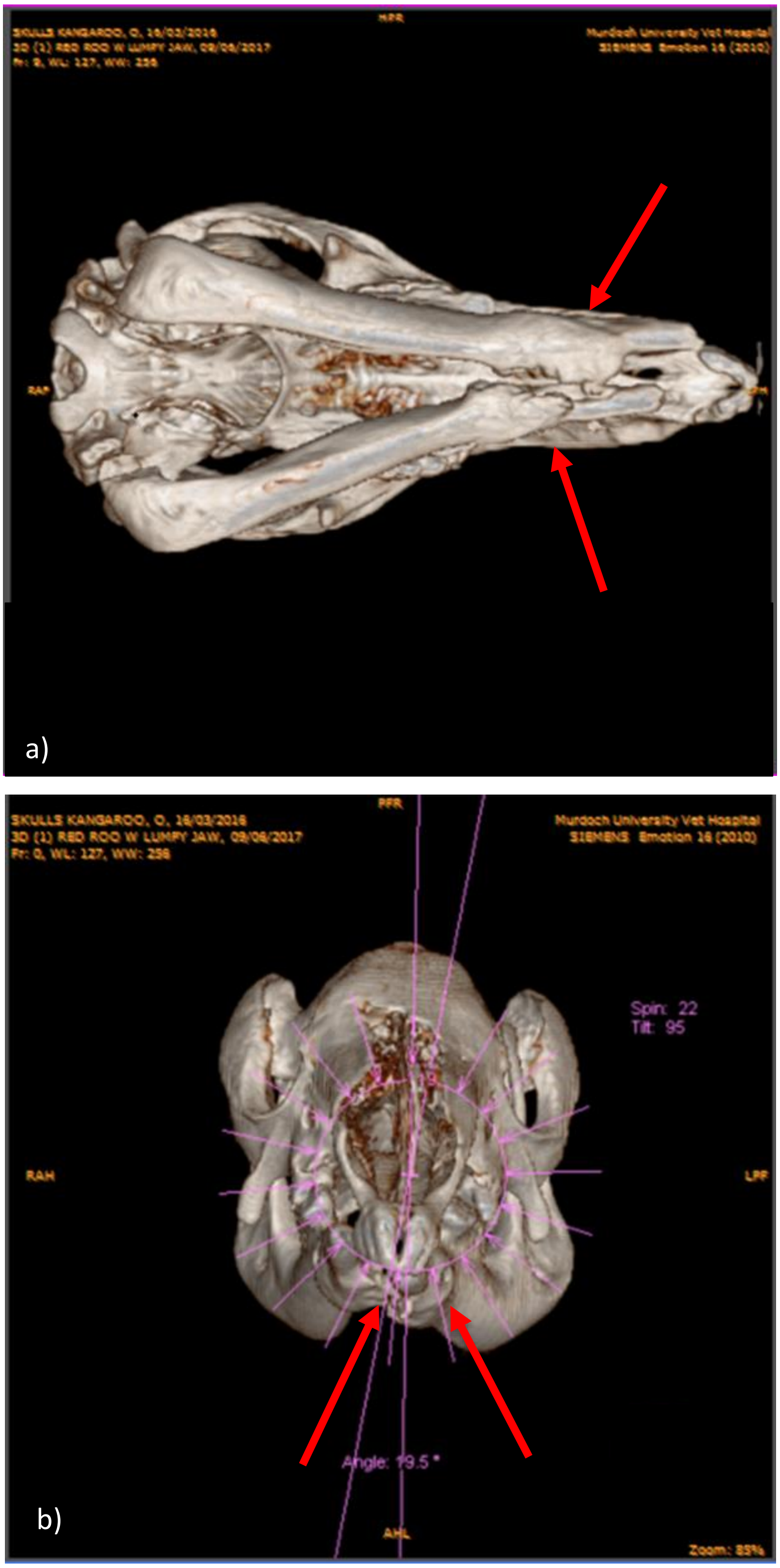

Figure 6.4: CT of the ventral view of a red kangaroo skull with confirmed lumpy jaw. The mandible has bilateral proliferative lesions with intraosseous opacity (osteolysis) and new periosteal new bone formation creating expansile lesions (red arrows); a) transverse plane, b) frontal plane. Lesions not visible from dorsal view.

6.5 Discussion

The review of CT images and visual observation of the skulls from this sample of western grey kangaroos did not detect osteolytic changes consistent with lumpy jaw. All available bones of the skull and jaw bones were reviewed, and results presented a prevalence of 0% (95% CI: 0.0 - 2.9). These results support our hypothesis that the prevalence of lumpy jaw is lower in isolated wild western grey kangaroos than the prevalence previously reported in captive conspecifics (2.6%) (Vogelnest & Portas, 2008). The absence of lumpy jaw in this sample population does not confirm that the disease was not present in the wider population. However, we would expect that lumpy jaw disease, if present in the overall population, would also have a low prevalence.

6.5.1 Prevalence

Our results support the prevailing body of literature that suggests that the prevalence of lumpy jaw is lower in wild kangaroos than that observed in captivity (Wallach, 1971; Butler, 1981; Vogelnest & Portas, 2008). Vogelnest and Portas (2008) reported a low prevalence of the disease in captive western grey kangaroos of 2.6%. In comparison, the prevalence of lumpy jaw reported at the same institution for another species, the red kangaroo, was 29.6% (Vogelnest & Portas, 2008); considerably higher than that reported in the western grey kangaroo. This could indicate support for the Calaby and Poole (1971) hypothesis of species specificity. However, the results presented in these captive studies were based upon findings at necropsy, and do not include animals successfully treated for the disease; meaning the figure presented was likely skewed.

6.5.2 Diagnostic methods

Detection of disease in wild animals often involves visual observation of noticeable behavioural or physical abnormalities, or locating and sampling dead individuals (Thomas et al., 1997; Hartup et al., 2001; Borland et al., 2012). Visual observation of lumpy jaw is subjective, and without the support of other diagnostic tools it is difficult to confirm a definitive diagnosis. The Tomlinson and Gooding (1954) study discussed jaw and leg swellings, indicative of osteomyelitis, in addition to observed emaciation, yet it was not clear if the jaw swellings were cases of lumpy jaw. A diagnosis of lumpy jaw can be confirmed through clinical and radiographic examination, biological sampling and necropsy. Our methods followed those of Miller and Beighton (1979) and Borland et al. (2012). We initially used visual observation of the specimens to detect evidence of proliferative bony changes and this disease was not detected. Visual observation of osteolytic changes is based on the skill of the observer. The development of clear diagnostic criteria may provide a more objective approach to this method of reporting, similar to that used by Borland et al. (2012). In contrast, in our study, and in that of Miller and Beighton (1979), only lesions that appeared proliferative were considered to be cases of lumpy jaw. This reduced the risk of misrepresenting (over-representing) the prevalence of the disease. Lumpy jaw is syndromic, and it has been suggested that the disease requires not only the presence of proliferative bony change, but also soft tissue inflammation (Vogelnest & Portas, 2008). There are obvious limitations to the use of skulls, without the presence of soft tissue, to describe the prevalence of this disease. This highlights the need for development of a clear definition of the physiological and biological factors required to represent a true case of lumpy jaw, to clarify this matter.

To confirm the presence and extent of bony involvement in lumpy jaw, diagnostic imaging is recommended (Miller et al., 1978; Miller & Beighton, 1979; Vogelnest & Portas, 2008). Plain radiography is typically used for this purpose (Jackson, 2003), and was the diagnostic tool of choice for Miller and Beighton (1979). Computed tomography is another more advanced option that has reliably been used in the diagnosis of lumpy jaw in kangaroos (Melbourne Zoo, 2007; Lee et al., 2011). Its major advantage over radiography is that of superior detail of both soft tissue and bone, which may aid in the diagnosis of mild cases (Lee et al., 2011). However, due to the costs of CT imaging, it is typically only available at large university teaching hospitals or through specialist referral to veterinary and/ or human radiology practices, and so is not always a practical option and precludes its use in veterinary medicine.

Detection of lumpy jaw in wild populations is also limited due to the flighty nature of macropods and the consequent need for chemical immobilisation to enable clinical examination. Therefore, disease detection in wild individuals frequently relies on examination of cadavers, for which soft tissue may have undergone autolysis, or may not even be present, limiting the extent of analysis possible with CT. In these circumstances, radiography may be of equal value, and potentially easier to access. In this current study, CT was used successfully to detect bony proliferations and osteolytic changes in the control specimen. In the study specimens (the western grey kangaroos), all areas of the mandible, maxilla and the skull were examined, and no signs of osteolysis or bony proliferation were detected in the population from either study site, therefore no cases were detected. It needs to be noted that the specimens examined were skulls only, without the presence of soft tissue, and therefore we cannot rule out the possibility of mild lumpy jaw cases involving soft tissue involvement only, in this population. However, it seems unlikely that such mild cases would not progress to severe cases in a wild population, where individuals would not be receiving treatment.

Additionally, 18.9% of the specimens presented for CT were incomplete, having either one hemi-mandible or large section of the rostral mandible missing. Borland et al. (2012) reported that the greatest number of proliferative lumpy jaw lesions occurred in the rostral region of the mandible; so it cannot be ruled out that the low prevalence observed in the current study may be related to the missing bone matter from these regions. Although our sample size (n = 127) exceeded the requirements from a power analysis of a minimum requirement of n = 121, the total number of complete specimens (n = 103) was below this requirement. In addition, information regarding the size of the original populations at the source was not certain; therefore, the sample size obtained (n = 127) for the two locations may not be sufficient for the true prevalence in the populations to be determined.

6.5.3 Risk factors for lumpy jaw

Several macropod species undergo the process of molar progression, however the replacement and timing of molar progression is species-specific (Jackson, 2003). Molar progression is age-related, and has been proposed to be associated with the development of lumpy jaw due to two hypothesised factors: (i) breaches in mucosa associated with molar progression allow points of entry for bacteria, as previously postulated (Finnie, 1976; Arundel et al., 1977); and (ii) the 'post-functional' molar teeth create a trap for particles of food and pathogens, resulting in opportunistic infection (Miller & Beighton, 1979). If molar progression is indeed a factor, many of the individuals examined in our study may have been too young to show evidence of lumpy jaw. We also note that the mean age of individuals in our study was younger than those individuals presenting with proliferative lesions in the Borland et al. (2012) study. However, Borland et al. (2012) observed that bone lesions were usually involved with the more rostral teeth found within the oral cavity, rather than with erupting molars; suggesting that the eruption of molar teeth may not be a common predisposing factor in lumpy jaw.

Osteolysis, defined as the pathological process of the destruction or reabsorption of bone tissue (Agarwal, 2004), is associated with both lumpy jaw and periodontal disease (Miller & Beighton, 1979; Clarke, 2003; Gamble, 2004). Periodontal disease is a condition that is considered to be a precursor for lumpy jaw (Burton, 1981; Borland et al., 2012) and is also associated with dietary products being impacted between the teeth. In conjunction with the development of dental calculus, periodontal disease may lead to gingival recession and alveolar bone loss (Miller & Beighton, 1979; Clarke, 2003). However, no bone loss was detected in our sample population, which may suggest that the diet in our study population differed from that in the population studied by Borland et al. (2012). The Borland et al. (2012) study was carried out in the 250 hectare Serendip Wildlife Sanctuary, Victoria, at a time of extreme drought. In contrast to the present study, where grazing was abundant, grazing material in the Serendip Wildlife Sanctuary was considered dry and to be in short supply. In addition, the pastureland was heavily contaminated with faecal matter due to the increase in population density around limited grazing material. In these specific circumstances, kangaroos may have had a greater predisposition to developing lumpy jaw for several reasons: a) being at increased risk of exposure to, and ingestion of, pathogenic bacteria during grazing upon contaminated pasture (Smith et al., 1984; Smith & Thornton, 1993; Vogelnest & Portas, 2008); b) enduring oral trauma whilst grazing upon dry, coarse grazing material, as oral trauma often precedes infection (Finnie, 1976; Gamble, 2004; Vogelnest & Portas, 2008); and c) overcrowding may have led to competition over resources, stimulating stress-induced immunosuppression (Blecha, 2000). Further investigations of wild kangaroo populations may determine if a disease incidence significantly increases under environmentally challenging conditions.

Diet has an influence on macropod dentition, and on oral health and associated risk of disease (Clarke, 2003). Macropods are categorised by dietary preference: as grazers, browsers or mixed grazer/browsers; and the western grey kangaroo is predominantly a grazer (Sanson, 1989; Tyndale-Biscoe, 2005; Arman & Prideaux, 2015). The species feeds principally on grass, a product which is largely unvarying and is abrasive in its physical properties (Hume, 1982; Johnson-Delaney, 2014). Grass was the predominant substrate at both study sites, Melville Glades and Thomson's Lake Reserve, and it could be suggested that the abrasive nature of this product on the dentition may lead to a lower incidence of lumpy jaw by facilitating appropriate dental wear as suggested by Sanson et al. (2007). Sanson et al. (2007) suggested it was the abrasive nature of silicacious phytoliths in grasses that caused tooth wear, and the long-term effects of abrasiveness of dietary products on teeth has also been observed in another herbivorous species, the giraffe (*Giraffa camelopardalis*) (Clauss et al., 2007). The effect of different diets on dental anatomy led to a different classification in this species, with wild giraffe classified as browsers and captive conspecifics as grazers; thus demonstrating the influence of diet on dentition.

The area in which the kangaroo specimens were sourced for this study is naturally sandy (Perth Metropolitan Region, WA), therefore, the suggested abrasiveness may not be caused by the grasses, but by silica contamination from the natural habitat (Kaiser et al., 2009). Further research into the effects of different substrates ingested by wild kangaroos, and any association with the prevalence of lumpy jaw, is recommended. The results of such research could benefit wild and captive macropods alike, by increasing our knowledge of the potential aetiology of this refractory disease. Recommendations could be developed, based on the diet of wild conspecifics, regarding the feeding of specific dietary products that may reduce incidence of lumpy jaw in captive populations, where this disease is of major concern (Vogelnest & Portas, 2008).

We also considered any relationship between prevalence of lumpy jaw and overpopulation. When western grey kangaroos experience overpopulation or overcrowding that causes declines in their preferred diet (graze), the species is able to obtain nutrients instead through the ingestion of shrubs and browse (Barker, 1987; Munn et al., 2014). Reports taken at the time of specimen collection for this study, however, suggest that conditions at the time were favourable, and the preferred diet was adequate (Mayberry, 2009). This may have contributed to the reported healthy, potentially overpopulous, kangaroo population at the study sites (Mayberry, 2009). The kangaroo populations at both sites were culled as they were beginning to have an impact upon the vegetation. Overcrowding and a reduction in resources can be associated with lumpy jaw, as demonstrated by Borland et al. (2012); yet despite the relatively high densities reported for our study populations, a lack of cases was detected. Although the exact population densities and food abundance are unknown, it is likely that there was still sufficient vegetation to support the population in both these localities where samples were collected 10 years prior.

Environmental contamination and presence of pathogenic bacteria were also examined as a potential factor affecting prevalence of lumpy jaw. Overpopulation and the forced congregation of animals can have a profound effect on the presence of disease (Wobester, 2006), and is noted as a specific risk factor for lumpy jaw in captive macropods, specifically in the presence of pathogenic bacteria (Ketz, 1997). *Fusobacterium necrophorum* is a primary causative bacterium of lumpy jaw (Burton, 1981; Samuel, 1983; Vogelnest & Portas, 2008), and is also a species previously found to be associated with environmental factors such as overcrowding, subsequent faecal contamination and footrot in domestic livestock (Bennett et al., 2009). Footrot is an infectious bacterial disease known to be transmitted via contact with, or ingestion of, contaminated material (Whittier & Umberger, 2009). When circumstances permit, kangaroos that share their environment with infected livestock could be at increased risk of lumpy jaw through the ingestion of pathogenic bacteria, particularly when grazing (Burton, 1981; Bennett et al., 2009). However, the kangaroos in the Thomson's Lake Reserve were completely contained, preventing any possible contact with domestic livestock. Those at Melville Glades had the capacity to disperse via the main driveway of the golf course, but appeared to remain within the perimeter fencing. Further, Melville Glades is located in an urban area where livestock are absent. Although studies have reported a high prevalence of lumpy jaw when kangaroos share their environment with domestic livestock (Tomlinson & Gooding, 1954; Borland et al., 2012), the absence of contact between kangaroos involved in our study and livestock (Mayberry, 2009) may help explain the low prevalence of lumpy jaw observed. However, prevalence of lumpy jaw is high in captive populations (as reported in Chapters 3 and 4), where exposure to domestic livestock is also absent. Population density, for both wild and captive macropods, may affect levels of environmental contamination and the development of lumpy jaw. Further investigations into potential links between lumpy jaw and environmental contamination are recommended.

6.5.4 Limitations of this research

Research using specimens collected historically presents difficulties, and retrospective analysis of environmental risk factors is particularly challenging. An additional complication in our study was that a number of the specimens were incomplete, including missing sections of the jaw most associated with lumpy jaw lesions (the rostral mandible) (Borland et al., 2012). The absence of soft tissue, which is required for confirmation of the presence of lumpy jaw in live macropods, may also have influenced the lack of lumpy jaw cases that we observed. Additionally, the lack of previous research into lumpy jaw in wild populations of kangaroos meant that the evaluation of risk factors was limited.

6.5.5 Recommendations and future research

This study reported prevalence of lumpy jaw in a sample population of western grey kangaroos; however, we encourage further investigation into disease detection and the characterisation of prevalence in other populations and species of wild macropods. It is challenging to detect and measure disease in wild animals (Wobester, 2006), especially during the early stages of disease where clinical and behavioural signs may not yet be obvious (Miller et al., 1978; Wobester, 2006). Lumpy jaw is a progressive disease, often initiated by periodontal disease (Clarke, 2003), progressing to inflammation of the soft tissue and in the later stages of the disease, it involves the bones of the mouth and jaw. Clinical analysis and confirmation of the early, and possibly latter stages of the disease in wild kangaroos would require direct handling of the animals; this can prove challenging, requiring immobilisation and veterinary support for diagnostics and recovery. Mayberry (2009) demonstrated that the western grey kangaroo can safely be sedated and examined in the field, including collection of oral biopsy samples and swabs for microbial culture to confirm a diagnosis of lumpy jaw. Although characterising this disease in the field would be challenging without the supportive radiographic evidence of bony involvement. Undertaking a larger study incorporating opportunistic data collection from specimens from road traffic collisions, as well as systematic collection of specimens in the field, could potentially provide a clearer indication of true prevalence in this species. Studies could extend further to examine environmental conditions, including quality, quantity and type of pasture/forage/browse on which the wild kangaroos feed. Better knowledge of the vegetation selected by wild macropods could be used to inform captive collection managers regarding the most appropriate browse and forage for this species in captivity. Given that lumpy jaw is widespread in captive settings, this information could be valuable for disease risk management. Additionally, a collaborative approach to disease surveillance should be encouraged, with wildlife managers, professional shooters, abattoirs and pastoralists working together to record health and disease status of macropod populations. Knowledge gained regarding existing population health and demographics could aid the future management of kangaroo populations, with information gained benefitting both captive and wild macropods.

Given that the prevalence of lumpy jaw in the western grey kangaroo appears to be low in captivity, and was undetected in this wild population, further investigations into species specificity are recommended. A potential risk factor for lumpy jaw involves the impaction of food around the teeth and gums caused by the narrowness of the jaws (Crandall, 1964); an aspect of macropod anatomy that is variable across Macropodidae (Jackson, 2003). Anatomical differences in dentition could be the basis for a species-specific susceptibility (Calaby & Poole, 1971). Investigations into species-specific prevalence and anatomical risk factors could benefit those who manage kangaroos in the wild and in captivity, and as such are highly recommended.

6.6 Conclusion

This epidemiologic study investigated the prevalence of lumpy jaw in two isolated wild populations of western grey kangaroos using CT as a diagnostic tool. Lumpy jaw is a chronic condition that often leads to malnutrition, starvation and death, however this disease was not detected in these populations of western grey kangaroos. Further surveillance of wild kangaroo populations would assist with determining species susceptibility and improve our understanding of the biological significance, development and potential spread of lumpy jaw. Lumpy jaw continues to be a problem of captive macropods, and there is interest by zoological institutions in investigations from wild populations, to gain knowledge of disease prevalence in the natural environment and a better understanding of risk factors associated with the development of lumpy jaw in captive populations.

Chapter 7

General Discussion

7.1 Summary of observations and hypotheses

This study is the first systematic study of lumpy jaw in captive macropods from multiple institutions across the Australian and European regions, providing epidemiological data and risk factor analyses for the disease. This research aimed to investigate temporal and spatial distribution of lumpy jaw in captive macropods and review risk factors for the disease. The research used epidemiological methodology, including a cross-sectional survey of zoological institutions across the Australian and European regions, a detailed retrospective cohort study of zoo records, and an examination of lumpy jaw in isolated wild populations of macropods. Collectively, the findings from this study provide evidence in support of previously hypothesised risk factors, such as stress (Finnie, 1976; Vogelnest & Portas, 2008; Borland et al., 2012), genera- and species-specific susceptibility (Calaby & Poole, 1971), which may be associated with the development of the disease. Our results also provide evidence that macropod sex and age, as well as reduced anthropogenic-related biosecurity within macropod enclosures, are significant factors associated with development of the disease; however, regional bias exists and this requires further examination.

7.1.1 Prevalence and incidence

Prevalence and incidence are useful measures of disease in animal populations, and study design plays an important role in facilitating the collection of data relating to disease burden and causality (Thrusfield & Christley, 2018). Prevalence estimates from this study's survey provide an indication of the importance of lumpy jaw to macropod collections across several countries, and this cross-sectional study has enabled us to detect lumpy jaw in countries not previously reported. Prevalence, as determined from this study's survey, showed both a country-level and regional effect, with macropods housed in European institutions identified to be at increased risk of developing the disease than those in Australia. This supports suggestions of climatic influence on the occurrence of lumpy jaw (Oliphant et al., 1984; Kido et al., 2013). This is contrary to the results from the retrospective cohort study, where lumpy jaw across the two regions appeared to be of similar prevalence, although a longer time period was examined. Study design did not appear to have an effect on prevalence estimates for the Australian region in particular, where figures reflect those previously presented by Vogelnest and Portas (2008). However, it is highly likely that there were more cases of lumpy jaw in Europe in the early years of the retrospective study, but the limited records denied us the opportunity to investigate this further, and may have resulted in the difference in reported prevalence observed between the two epidemiological studies conducted in Chapters 3 and 4.

Retrospective studies may be the subject of a selection bias (Thrusfield & Christley, 2018), and based on potential species-susceptibly to the disease (Calaby & Poole, 1971), selection bias may also be present in the retrospective cohort study undertaken as part of this research. Institutions were initially selected for the presence of the western grey kangaroo, a species with a reported prevalence of lumpy jaw of 2.6% (Vogelnest & Portas, 2008). Results from this research report zero to low prevalence of lumpy jaw in this species, both in captivity, and as anticipated, in the wild (Wallach, 1971; Miller & Beighton, 1979; Vogelnest & Portas, 2008). The presence of species with low susceptibility may result in the calculation of lower prevalence estimates that are not truly representative of the extent of lumpy jaw in captive macropod populations. Although species susceptibility requires further research, these data suggest that perhaps the captive management of macropod species of low susceptibility to lumpy jaw may be beneficial in reducing incidence of lumpy jaw in zoological collections.

Although prevalence is a useful measure in its own right, incidence rates provide a more accurate reflection of the level of risk (Thrusfield & Christley, 2018). Yet contrary to our expectation, incidence of lumpy jaw across our study was low. The retrospective investigation of zoo records found regional incidence rates were similar, indicating macropods from both the Australian and European regions were at similar risk of developing lumpy jaw. Although this similarity mirrors the prevalence results calculated from zoo records, the effect of missing zoo records for macropods housed in Europe in the earlier period is evident in the regional incidence rates presented (1995 - 1999). During this period, confidence intervals showed that there was a significant difference in incidence of lumpy jaw between the geographic regions (Australia IR 5.5, 95% CI: 4.1 – 7.2; Europe IR 1.1, 95% CI: 0.1 – 3.9). Consequently, we may reject hypotheses of a climatic influence on incidence of lumpy jaw (Oliphant et al., 1984; Kido et al., 2013). Regional differences were detected; however, they were limited to specific risk factors, and confounded by institutional differences in species housed, housing systems used, and husbandry practices used to manage macropods. The specific multivariate epidemiological analyses undertaken in this research are rare in studies of wildlife, and the results presented provide robust evidence of both animal-centred and environmental risk factors for lumpy jaw.

Although geographical region was not associated with the development of lumpy jaw, the distinct institutional differences in incidence of the disease warrants further investigation. The zoo identified as Zoo A3, had a significantly lower IRR than all the other Australian institutions. Undertaking a more detailed investigation of institutional practices across these institutions may identify aspects of captive housing and husbandry that correlate with the development of lumpy jaw. A well-designed longitudinal study, controlling not only for aspects such as substrate, population density and dynamics (including age and sex), would also be beneficial in clarifying any possible relationship between the captive environment and lumpy jaw.

7.2 Analysis of risk factors for lumpy jaw

7.2.1 Age

Results from this research determined that age is a risk factor for lumpy jaw, with an incremental increase in risk observed as macropods age. These findings support previous suggestions by Clarke (2003) and Kido et al. (2013), that lumpy jaw is associated with the age-related phenomenon, molar progression. Sanson (1989) adds specific detail to this argument discussing that uniform diets affect the pre-molars involved in molar progression, rendering them non-functional and resulting in dental problems. However, age-related dental development, and molar progression, are also influenced by the sex and species of macropod (Newsome et al., 1977; Jackson, 2003), and not all species experience molar progression (Jackson, 2003).

Zoo animals are now living longer in captivity, potentially due to improved veterinary care (Hosey et al., 2013). Yet, longevity also extends the time exposed to other risk factors for lumpy jaw, including stress (Vogelnest & Portas, 2008), and the longitudinal effect of diet on dentition (Glatt et al., 2008). In macropods, as in many other zoo animals, artificial or inappropriate diet may prolong tooth wear, subsequently affecting dental development (Glatt et al., 2008; Damuth & Janis, 2013; Hosey et al., 2013), and potentially increasing the risk of lumpy jaw. Although the age of onset of lumpy jaw varied between species, collectively the statistical model developed for this study determined that the oldest macropods (+10 years) were at greater risk of lumpy jaw when compared to juveniles (< 1 year). However, juveniles are still susceptible, as cases of the disease in juvenile macropods were identified in zoo records. Additionally, due to variation in life expectancy between macropod species (Jackson, 2003), it would be beneficial to identify a species-specific 'age of risk' by adjusting our statistical model to compare risks across other age categories, rather than just < 1 year olds, the reference category used in this study. Although these results suggest that increased longevity in captivity increases the risk of lumpy jaw, age is also a factor in many diseases (Thrusfield & Christley, 2018).

7.2.2 Sex

Animal sex may influence the development and outcome of disease (Hing et al., 2017). In this study there was a male bias in the incidence of lumpy jaw; however, this result was regionally biased. Male and female macropods are reported to differ in their feeding ecology, behaviour (Garnick et al., 2018; Rendle et al., 2018), and dental development (Newsome et al., 1977). For males, sex-specific aspects of their feeding ecology and behaviour (Jarman, 1984; Gansloßer, 1995; Rendle et al., 2018) may increase the risk of oral trauma, due to agonistic behaviour (Gansloßer, 1989), and influence the risk of developing lumpy jaw. Therefore, we expected to observe differences in risk between macropod sexes. However, many behavioural and anatomical features may also be species-specific; consequently, the regional skew observed in the analyses may be related to a greater presence of species that express these features within one region than another. Additionally, the regional sex-specific differences in risk of developing lumpy jaw, may be due to regional differences in the housing and management of male and female macropods; these differences may be a potential source of stress (Schulte-Hostedde & Mastromonaco, 2015; Rendle et al., 2018). Interestingly, the findings from our retrospective cohort study and from our cross-sectional survey found both geographic regions reported lumpy jaw more frequently in female macropods. Although further examination of the data could be conducted, the different results for the two studies within our overall research suggest that perhaps sex is not necessarily the driving factor in the development of lumpy jaw, as was also found by Vogelnest and Portas (2008), but perhaps it is the species that influences the risk of developing lumpy jaw.

7.3.3 Genus and species

Lumpy jaw was detected in all genera, and across methodologies, the most frequently reported cases were captured in *Macropus* and *Wallabia*. These results highlight that macropods classed as 'grazers', 'browsers' and 'mixed feeders' in their digestive strategy, are all susceptible to the disease. However, there are distinct categorical, anatomical and behavioural differences between genera and between species, which may influence the risk of lumpy jaw, and some of these risks may be associated with captivity. We anticipated greater incidence of lumpy jaw in *Macropus* than other genera, for two reasons: i) many species within this genus exhibit molar progression, a hypothesised risk factor for lumpy jaw (Clarke, 2003; Vogelnest & Portas, 2008); ii) many *Macropus* spp. are categorised as 'grazers' (Sanson, 1989; Arman & Prideaux, 2015), where tooth wear and the sequential progression of molar teeth may be particularly affected by an artificial diet (Burton, 1981; Lentle et al., 2003). The morphological effect of captive diets on zoo animal dentition has been recognised in the giraffe (*Giraffa camelopardalis*) (Kaiser et al., 2009), lion (*Panthera leo*) (Hollister, 1917) and cheetah (*Acinonyx jubatus*) (Fitch & Fagan, 1982), and especially where species are bred in captivity for several generations (Hartstone-Rose et al., 2014). The morphological effect of artificial diets in captive-bred macropods, in relation to incidence of lumpy jaw, requires further evaluation.

Potentially, genera- and species-specific susceptibility to the disease were identified in this research, whereby species within *Macropus*, such as the red kangaroo, appeared to be more susceptible to lumpy jaw. In addition, in our research, the western grey kangaroo was found to have a low prevalence in both captive and wild populations; thus indicating this species has a low susceptibility. The suggestion of species-specific susceptibility is further strengthened by the low incidence reported in the sole species in *Setonix*, the quokka. As a 'browser' species, molar progression is blocked in the quokka (Sanson, 1989; Jackson, 2003). Contrary to these hypotheses, a species that also does not exhibit molar progression, *Wallabia* sp. (swamp wallaby), was found to be at greatest risk of developing lumpy jaw in our research. This species has a reported history of high prevalence of lumpy jaw (Kido et al., 2013). These conflicting results raise questions about the association between molar progression and lumpy jaw, and we may seek explanations by examining the behavioural characteristics of macropods, such as their responses to stress. The flighty nature of some macropod species (Jackson, 2003) may for example, increase the risk of fence-running resulting in facial trauma, and may increase the immunosuppressant effects of stress (Dohms & Metz, 1991; Blecha, 2000), and thereby potentially increase the risk of lumpy jaw in those species (Butler, 1981; Vogelnest & Portas, 2008). However, stressors in captivity will arise from many sources (Morgan & Tromborg, 2007), which need to be individually examined to assess their impact or otherwise on macropod health and lumpy jaw in particular.

7.3.4 Stress

The suggestion that stress increases the risk of lumpy jaw in captive macropods (Finnie, 1976; Vogelnest & Portas, 2008; Borland et al., 2012) appears justified, given the strong evidence, identified in this research, of an association between a known stressor and the risk of developing lumpy jaw. Transfers between enclosures and/or zoos can involve exposure to many different sources of stress and health challenges for macropods, such as those gained from previous negative experience (Grandin, 1997); transfer frequency and duration (Dembiec et al., 2004; Broom, 2005; Padalino et al., 2015); isolation from the mob (McKenzie & Deane, 2005); overcrowding (King & Bradshaw, 2010; Borland et al., 2012) and exposure to pathogens (Reiss & Woods, 2011; Wildlife Health Australia, 2018). Transporting animals between locations also poses a biosecurity risk (Reiss & Woods, 2011; Wildlife Health Australia, 2018), through the transfer of pathogenic bacteria between locations, for which macropods may lack the acquired immunity from previous exposure (Mathews et al., 2006).

Primary pathogens identified by survey respondents differed between the two regions investigated in our research. Although macropods are not necessarily more susceptible to the pathogen most frequently reported in Europe (*Fusobacterium necrophorum*) (Smith et al., 1986), it is unknown if macropods have an increased susceptibility to a greater presence of *Pseudomonas aeruginosa*, the bacterial species most frequently reported in the Australian region. The increased odds of lumpy jaw found in Australian-housed macropods, compared to those housed in Europe, may therefore be the result of a combination of differences in transfer frequencies, distances, and potentially a susceptibility and exposure to pathogens. These combined factors might result in macropods in Australia being particularly vulnerable to stress-induced immunosuppression, as previously reported for a number of Australian marsupial species such as the woylie (*Bettongia penicillata*) (Hing et al., 2014) and the tammar wallaby (McKenzie & Deane, 2005).

7.3.5 Biosecurity

Ensuring good hygiene within macropod enclosures is a long established recommendation in the control of lumpy jaw (Calaby & Poole, 1971; Finnie, 1976; Butler & Burton, 1980; Burton, 1981; Ketz, 1997; Vogelnest & Portas, 2008); however, our findings suggest that the source of contamination is anthropogenic. We present empirical evidence that a lack of personnel biosecurity, relating to footwear, tools and equipment used around macropod enclosures, substantially increases the risk of lumpy jaw. The survey undertaken as part of our research demonstrated keeper and tool biosecurity were not a priority for the majority of zoos, and personal observation of institutional protocols found that strict biosecurity protocols around keeper footwear, tools and equipment used in animal enclosures, were not routinely in use. However, the source of enclosure contamination may also originate from zoo visitors entering walk-through enclosures, or even from the animals themselves. Disease may also be spread during transport (Fèvre et al., 2006; Padalino et al., 2015), and the transfer of macropods between enclosures, and zoos, may increase the risk of bacterial contamination. Additionally, animals that are frequently transferred are more likely to be immunosuppressed though the stressors associated with translocations. The increase in incidence of lumpy jaw in macropods that had experienced several inter- and intra-zoo transfers supports these suggestions. Therefore, we recommend that a review of biosecurity measures surrounding inter- and intra-zoo transfers is undertaken, and that adequate biosecurity protocols are followed during and after all macropod transfers. Control of environmental bacteria is essential in the prevention of disease (Bennett et al., 2009; Wildlife Health Australia, 2018), and control measures may influence the efficacy of treatment, for example, the infection of a wound site due to bacterial contamination, resulting in possible disease recurrence. Therefore, minimising bacterial presence is essential. Historically environmental biosecurity efforts have centred on raising feeding platforms to prevent faecal contamination of feed and this was part of a preventative strategy that was implemented at Zoo A3 in 2002 (B. Laming, personal communication, 12th June 2017). In comparing institutional incidence rates across the Australian region, our data indicates that for Zoo A3, this may potentially have been an effective strategy. Further examination of the data, reviewing incidence rates over time for this particular institution, may ascertain if these specific changes had any real effect on incidence of lumpy jaw.

7.3 Treatment and outcome

Treatment for lumpy jaw remains a challenge, and findings from this research suggest that irrespective of treatment approach, recurrence of the disease is frequent and mortality is common. The regional preference for a specific treatment approach was potentially influenced by regional differences in presence of veterinary facilities and personnel. Both the survey-based study and the retrospective study in our research indicated that European institutions had less veterinary support than Australian institutions. However, macropods that have surgical intervention, are arguably more likely to be suffering from a severe case of lumpy jaw, requiring radical treatment. Therefore, that particular individual may perhaps also be more susceptible to recurrence as a result of the completeness (or otherwise) of surgical debridement and tooth extraction, for example. Recurrence may also be affected by the efficacy of antibiotic therapy. Limitations associated with unknown pharmacokinetics and pharmacodynamics in macropods, include incomplete penetration into necrotic and abscessed tissue, and should be considered. Post-treatment hygiene may also affect the likelihood of reinfection, and maintaining a hygienic post-surgical environment is challenging, especially given the presence of environmental bacteria and the potential for contamination by zoo personnel, and potentially zoo visitors. Different bacterial species were identified in Australia than in Europe; therefore, bacterial culture and sensitivity testing is recommended to ensure an appropriate antibiotic is selected. Treatment for lumpy jaw can be traumatic, both in terms of the impact upon tissue and, with regard to increased stress for the individual macropod. Treatment should only be continued if pain and infection can be controlled and stress during treatment can be minimised. It is important to weigh up the welfare implications of treating the disease against the likely outcome; and future efforts should focus primarily on the prevention of lumpy jaw rather than the cure.

7.4 Effects of treatment over time

Even with improvements in veterinary knowledge over time, this research has demonstrated that lumpy jaw is a disease of similar magnitude today as it was 20 years ago. New treatments for lumpy jaw have been reported in recent years, for instance, chlorhexidine varnish (Bakal-Weiss et al., 2010), however, their efficacy is yet to be scientifically tested. Furthermore, results from this research have potentially revealed that the husbandry recommendations made by Burton (1981), have either not been implemented, or were ineffective in the control of lumpy jaw in captivity. However, institutional differences in husbandry practices, and the personnel that deliver them, will undoubtedly affect the presence of diseases such as lumpy jaw.

7.5 Effects of institutional differences on the development of lumpy jaw

All known risk factors for lumpy jaw are related to institutional differences in housing, husbandry and species housed, and teasing out the specific factors in a multi-institutional study was challenging. Institutional results, specifically in the Australian region, have highlighted that Zoo A3 has a significantly lower incidence of lumpy jaw than the other Australian institutions sampled in this research, which may be related to the particular species managed, or the ways in which they are managed. Results for Zoo A3 are interesting, and further examination of the institutional practices, and special attributes that may have led to this result should be a priority for further research.

7.6 Limitations associated with this research

Study-specific limitations have been discussed in the relevant chapters, however some overarching limitations require consideration, including: our definition of lumpy jaw; the accuracy of records used in the study; and sample sizes, particularly with regards to species data.

7.6.1 Definition

The development of a case definition for lumpy jaw was essential for this research, to ensure the syndromic nature of lumpy jaw could be captured in case reports, using standardised methodology. Our definition aimed to retrospectively capture cases that fitted with a clinical description of the disease provided by Vogelnest and Portas (2008), and/or medical terminology for the condition provided in the records (oral necrobacillosis, mandibular/maxillary osteomyelitis). Reliance was therefore placed upon the details recorded at the time of diagnosis, which were sometimes ambiguous. Although the definition developed for this research encapsulated both the soft tissue and bony elements of the disease, it was not sensitive enough to detect early stages of the disease when lumps were not visible, for instance, gingivitis, gingival pocketing and tooth root abscess. Arguably, these early stages are conditions in their own right, and can occur without progression into lumpy jaw. An important consideration is that the case definition was developed to retrospectively capture cases of lumpy jaw, using clinical notes and diagnostics in zoo records, therefore, our definition may differ from what an ideal case definition would be if a full diagnostic work up was performed. Syndromic surveillance of lumpy jaw in captive macropods, using clinical and pathological characteristics of the condition as suggested by Ryser-Degiorgis (2013), would assist in defining the disease further.

7.6.2 Record accuracy

Records are vital for documenting the health of individuals over time, and are an essential tool for retrospective research such as this. However, the reliability of records relies greatly on the accuracy and detail of record content, either as entered, or potentially as affected by computer-based glitches or viruses; which are factors that researchers are unable to control for, or often even detect. Our examinations of institutional differences in the use of ZIMS, and subsequent record management, found that some institutions maintained detailed, longitudinal records, whilst others held records that were variable, both in depth and detail. The differences between record keeping may have affected the data collected during the retrospective investigation, and also the data collected by those that completed the survey. Additionally, a variety of zoo personnel entered data into ZIMS, increasing the risk of errors or omissions in the records. This was notable on occasions where clinical information was omitted from veterinary records; in one example, the delivery of medical treatment for lumpy jaw was detected in the husbandry section, an area of ZIMS predominantly used by keepers, yet there was no record of product or dose in the medical records. To counteract this issue during our research, all available records for all individuals were downloaded and reviewed, despite which, collectively, records varied in their detail. This highlights the need for zoos to employ a registrar responsible for accurate recording of animal data within the zoo, and auditing records to ensure they are detailed enough for future referral.

7.6.3 Sample size

Small sample size, particularly when examining the data at species level, limited statistical extrapolation, by reducing precision in prevalence estimates and measures of effect. Small sample size is a common problem with zoo-based and wildlife research (Steidl et al., 1997; Hosey et al., 2013); in this study, attempts were made to manage this issue by including multiple institutions and by using an expansive retrospective period. In addition, we conducted power analyses based on an unknown sample size, with sensitivity and specificity values that were reliant on both the accuracy and presence of zoo records. In the absence of some zoo records, an inability to correctly identify all the non-diseased macropods affected the specificity – the true negative rate. Nevertheless, when power analyses were conducted retrospectively, working with the assumption of sensitivity and specificity both at 0.9, and with an assumed prevalence of 10% (0.1) with 95% confidence, the minimum target sample size of 335 was exceeded.

7.7 Future research

Although this research has identified patterns, distribution and risk factors for lumpy jaw, this research has also raised several questions that require further investigation, as discussed below. Further research is also required to clarify or expand on findings from this study.

7.7.1 Potential effect of captivity and artificial diet on dental morphology

Various morphological effects of captivity in zoo animals are well reported (Caumul & Polly, 2005; O'Regan & Kitchener, 2005; Christensen, 2014; Hartstone-Rose et al., 2014), in the context of our research, the longitudinal consequences of captivity and captive diets on dental morphology in macropods may potentially result in a greater susceptibility to lumpy jaw. The morphological adaptations of macropod teeth to specific diets have previously been examined by Sanson (1980; 1989), and more recently by Christensen (2014), however these studies focussed on the morphological effects of diet in wild macropods, where animals select a diet associated with the particular ecological niche to which they have adapted. Although zoos provide their animals with a diet that meets the nutritional requirements for the species (Hartstone-Rose et al., 2014), they are often challenged when attempting to ensure that captive diets meet other important criteria for the species, including key physiological and mechanical properties that are essential for the species. It is often the absence of these aspects of a natural diet that can affect the dental health, and dental morphology of animals in captivity (O'Regan & Kitchener, 2005; Glatt et al., 2008; Hartstone-Rose et al., 2014). In other herbivorous species, the effect of an inappropriate captive diet resulted in morphological changes in tooth wear patterns that subsequently led to a re-classification of the dentition; from that of a browser to that of a grazer (Clauss et al., 2007; Clauss et al., 2008). Generational breeding of macropods in captivity, especially where the captive population has not been supplemented with new bloodlines from wild populations, which would not be possible in Europe, and coupled with the long-term effect of artificial diets, may result in morphological changes to the teeth and jaw. However, relationships between the long-term effects of captivity, through generations of macropods, in relation to the development of lumpy jaw, is yet to be examined. Further studies that examine macropod lineage, diet and morphology, in relation to lumpy jaw should be a priority for future research.

7.7.2 Prophylactic and remedial treatment for lumpy jaw

Preventive vaccination

Primarily, future research efforts should be directed towards preventative measures, such as vaccination. Historically, vaccination programs for lumpy jaw have had mixed results (Blanden et al., 1987; Gulland et al., 1987), although this may be a consequence of protocols required to maintain a protective antibody response (Barnes et al., 2009). In more recent studies, Phillips et al. (2012) examined the immune response against the tetanus component of the Ultravac® 5 in 1 vaccine (Pfizer Australia Pty Ltd., West Ryde, NSW) in macropods, a vaccine used to prevent clostridial diseases in livestock. The study established repeated exposure and booster vaccinations stimulated effective immunological protection. Importantly, Phillips et al. (2012) provide evidence of variation in immune response to the vaccine between macropod species and ages, something that could be factored into future studies of vaccinations to control lumpy jaw. Zoos have historically used Footvax® to provide protection against the various strains of *Dichelobacter nodosus* (Jackson, 2003; Vogelnest & Portas, 2008). However, the efficacy of vaccinations in protecting against lumpy jaw in macropods, has not been examined, and as of 2008 this product has been withdrawn from the Australian market over concerns relating to the product's serum content, which was considered a Bovine Spongiform Encephalopathy (BSE) risk. Retrospective studies of zoo records could be used to identify the outcome of vaccination programs already in place in some zoos, examining protocols for boosters and efficacy in preventing lumpy jaw. Additionally, longitudinal prospective studies that examine immune response to specific pathogens associated with lumpy jaw, including the leading species identified in this research, *Pseudomonas aeruginosa* and *Fusobacterium necrophorum* are highly recommended. Such studies would facilitate efforts to develop species and age-specific vaccination regimes to provide effective protection against lumpy jaw in captive macropods.

Antibiotic efficacy

Antibiotic efficacy is influenced by the selection of an appropriate product, which in turn is based on the results from bacterial culture (Jackson, 2003; Vogelnest & Portas, 2008). However, during this research, it was noted that the majority of institutions did not undertake routine microbial culture prior to the selection of an antibiotic. Additionally, several antibiotic products were often incorporated into the treatment of a case of lumpy jaw, therefore it was difficult to determine how efficacious a product was in providing a clinical resolution, as previously highlighted by Butler and Burton (1980). Although several antibiotics have been recommended for the treatment of lumpy jaw (see Chapter 5, Table 5.2, p. 146), it would be of considerable benefit for clinicians to obtain knowledge about the safe and effective therapeutic management of lumpy jaw; therefore, pharmacokinetic and treatment trials in macropods are recommended.

7.7.3 Efficacy of biosecurity

The results from this research indicate that occurrence of lumpy jaw may be anthropogenically driven, as we established a significant association between the development of the disease and a number of keeper biosecurity measures. Anthropogenic spread of animal disease is reported in several species, including foot and mouth disease in cattle and sheep (Defra, 2011) and white-nose syndrome in bats (Frick et al., 2016), and zoos acknowledge they are also at similar risk (Reiss & Woods, 2011). In addition to controlling pathogens through good husbandry practices, zoos also encourage personnel to adopt appropriate personal hygiene practices, including those involving removal of contaminants from footwear and equipment, in an effort to reduce disease risk (Reiss & Woods, 2011). However, footbaths for example, are not usually an efficacious biosecurity method and may be a proxy for other biosecurity measures undertaken at the institutional level (Firestone et al., 2011). This may also explain the difference in the results we report for footwear and tool biosecurity. However, animal management practices, including those involving biosecurity, are often reliant upon tradition rather than science (Melfi, 2009), and as our results demonstrate, biosecurity practices, specifically, may not be efficacious in controlling diseases such as lumpy jaw. We therefore encourage investigations into biosecurity practices, specifically of an anthropogenic nature, involving zoo personnel and visitors that enter macropod enclosures. These studies should include a retrospective examination of personnel biosecurity practices in relation to disease occurrence, in addition to a prospective examination of the introduction of new biosecurity practices, such as the introduction of footbaths for staff and visitors entering macropod enclosures.

7.7.4 Species specificity

Sample size prohibited the examination of risk of lumpy jaw to species level, yet zoos would benefit from knowledge of species-specific susceptibilities to lumpy jaw, to inform decisions regarding risk reduction, provision of specialist care, and reduction of both financial and welfare costs; for example by avoiding maintaining susceptible species in captivity. The collection and examination of veterinary records from a larger number of zoological institutions could supplement the data already held, increasing our sample size at the species level, and may provide clarification regarding species-level specificities.

7.7.5 Studies of wild macropod populations

Studies of lumpy jaw across a range of species of wild macropod species would not only complement the results already reported in this research, but may also provide species-specific benchmarks for the disease for captive conspecifics. Surveillance of lumpy jaw in wild populations, utilising carcases from abattoirs and those harvested in the field by licenced shooters, roadkill, facilities that rehabilitate wildlife, and zoos that already contribute to disease surveillance in wild animals (Cox-Witton et al., 2014), could provide a cost-efficient method of opportunistically surveying for the disease in wild macropods. The identification of benchmarks for disease in various species, including at times when macropods are deprived of essential resources, may benefit zoos when selecting macropod species to maintain within their institutions. Additionally, where outbreaks of lumpy jaw occur in the wild, it would be of interest to investigate disease dynamics, including that of pathogen presence in sympatric/other species, including domestic livestock. Bacterial species identified in cases of lumpy jaw are also found in cases of lumpy jaw in cattle (Walker & McKinnon, 2002), and footrot in sheep (Witcomb et al., 2014). Knowledge of a possible pathogenic spread during an outbreak of lumpy jaw has both economic and welfare implications for the farming industry.

7.8 Key management recommendations

The following recommendations for the control and management of lumpy jaw in captive macropods, have been developed as a result of this research, and are supported by a review of scientific evidence.

Increase clinical examinations of the oral cavity as macropods age

- The frequency of clinical examinations, specifically those that involve the assessment of the oral cavity, should be increased as macropods age. This is especially important for individuals over the age of 10 years.
- Ensure that not only adult macropods but also juveniles are included in all routine examinations that involve assessment of the oral cavity.
- Undertake behavioural training with macropods to facilitate examination of the jaw, head, neck and oral cavity, without the need for general anaesthetic. This may reduce the need for stressful transfers to veterinary hospital for clinical examinations.

Reduce internal and external transfers

- We present evidence of the longitudinal effect of zoo transfers on macropod health, and therefore strongly recommend that both the frequency and duration of inter- and intra-zoo transfer be minimised.
- To reduce the potential stress associated with aspects of transportation, such as confinement (King & Bradshaw, 2010; Borland et al., 2012), any macropods that may need to be transported should be habituated to modes of transport by crate training and handling as a matter of routine, and /or the use of neuroleptic drug therapy.

- Minimise other potential sources of stress in captivity such as visitor proximity and presence.

Increase biosecurity measures

- Biosecurity measures should be increased in and around macropod enclosures, and training provided in its use. This includes the cleaning of tools and equipment after each use and between enclosures. The implementation of dedicated footwear for each enclosure, footwear cleaning or footbaths for keepers to use, and potentially the introduction of footbaths for visitors entering walk-through enclosures, is strongly encouraged.
- Use feed and water containers that can be disinfected using bactericidal products, and ensure routine procedures are in place, and conducted, for the thorough cleaning of all inanimate objects and surfaces with which macropods may come into contact.

Treatment for lumpy jaw

- Each case of lumpy jaw is unique, with respect to the individual macropod affected, case severity, lesion location and potentially the species of pathogenic bacteria. We therefore recommend that each case should be managed using a case-by-case strategy, commencing with microbial culture to assist in the selection of antibiotics.
- Post-treatment care should involve minimising pain and stress, and importantly, ensure high standards of husbandry and hygiene are maintained throughout the recovery period.
- Recurrence is to be expected, therefore, additional observations of the affected macropod should be implemented 12 months post-recovery to look for early signs of recurrence. Early intervention may reduce the need for a radical treatment approach, and may improve the outcome for the macropod.
- The presence of veterinary personnel and access to suitable facilities is essential for the diagnosis and treatment of lumpy jaw. Therefore, zoos are encouraged to increase their access to veterinary support where this is not already in place on a regular basis.

Maintenance of zoo records

- The ZIMS database is a powerful tool that provides access to information about the health, disease, husbandry, behaviour and welfare of zoo animals on a global scale; however, its value is subject to the quality of the information entered. Therefore, data entered should be accurate, clear and detailed. For a more streamlined approach to record keeping, we specifically recommend that: there is an agreed definition of terms used in records; drop down menus for ease of selection appropriate categories; training of personnel entering data; the same person is responsible for entering data. Many institutions still hold paper-based and other electronic forms of records, yet regardless of the method of record keeping undertaken by an institution, the accurate and thorough maintenance of zoo records is highly recommended.
- The use of epidemiological techniques for records-based research is underutilised in zoo-based research, and could be used to answer a number of health, welfare and conservation questions. Zoo records provide a powerful source of data, as this research has demonstrated, and their use in future research is strongly recommended.

7.9 Final comments and conclusions

Retrospective studies are important for conservation management, and data contained in zoo records have the capacity to inform captive animal managers about best practice to reduce diseases such as lumpy jaw; thereby improving the health and welfare of macropods and other captive animals housed in zoological institutions across the world. This research provides novel and important information regarding the risks associated with the development of lumpy jaw, treatments for the disease and expected outcomes and recommendations for the management and control of clinical lumpy jaw, based on host and environmental factors associated with the development of the disease.

To maintain macropods in zoological collections is a privilege, with benefits including raising awareness of and encouraging an interest in native Australian wildlife. However, aspects of captivity have adverse consequences that result in diseases such as lumpy jaw. Zoos should be encouraged to minimise the risks associated with developing the disease, as identified in this research. Despite best efforts to treat this refractory disease, mortality rates remain high. The adverse consequences of lumpy jaw on captive macropod health and welfare deserve attention, and efforts should be directed towards future research that seeks to find preventative measures of controlling and managing this often-fatal disease. This is essential in order to achieve optimum health for captive macropods, to conserve species, and to provide supplementary populations of Australia's iconic animals for the future.

REFERENCES

Addy, L. D. & Martin, M. V. (2005). Clindamycin and dentistry. *British Dental Journal, 199*(1), 23-26. doi:10.1038/sj.bdj.4812535

Agarwal, S. (2004). Osteolysis - basic science, incidence and diagnosis. *Current Orthopaedics, 18*(3), 220-231. doi:10.1016/j.cuor.2004.03.002

Agarwal, S. & Chandra, A. (2014). Case of lumpy jaw in cross breed cow. *Indian Cow (The): The Scientific and Economic Journal, 10*(39), 2-4.

Alexander, J. E., Colyer, A., Haydock, R. M., Hayek, M. G. & Park, J. (2018). Understanding how dogs age: Longitudinal analysis of markers of inflammation, immune function, and oxidative stress. *The Journal of Gerontology: Series A, 73*(6), 720-728. doi:10.1093/gerona/glx182

Antiabong, J. F., Boardman, W., Moore, R. B., Brown, M. H. & Ball, A. S. (2013a). The oral microbial community of gingivitis and lumpy jaw in captive macropods. *Research in Veterinary Science, 95*(3), 996. doi:10.1016/j.rvsc.2013.08.010

Antiabong, J. F., Boardman, W., Smith, I. & Brown, M. H. (2013b). A molecular survey of a captive wallaby population for periodontopathogens and the co-incidence of *Fusobacterium necrophorum* subspecies *necrophorum* with periodontal diseases. *Veterinary Microbiology, 163*(3-4), 335. doi:10.1016/j.vetmic.2013.01.012

Arman, S. D. & Prideaux, G. J. (2015). Dietary classification of extant kangaroos and their relatives (Marsupialia: Macropodoidea). *Australa Ecology, 40*(8), 909-922. doi:10.1111/aec.12273

Arundel, J., Barker, I. K. & Beveridge, I. (1977). Diseases of marsupials. In B. Stonehouse & D. Gilmore (Eds.), *The biology of marsupials* (pp. 141-154). London: Macmillan Press.

Asperger, M., Thielebein, J., Selbitz, H., Scheller, R. & Eulenberger, K. (2001). *Investigations into the aetiology of lumpy jaw in macropods.* Paper presented at the Erkrankungen der Zootiere: Verhandlungsbericht des Internationalen Symposiums über die Erkrankungen der Zoo-und Wildtiere, Rotterdam, The Netherlands, 23-26 May (p. 251) Leibniz, Germany: Institut für Zoo-und Wildtierforschung.

Bailie, C. L., Baxter, M. & O'Connell, N. E. (2018). Exploring perch provision options for commercial broiler chickens. *Applied Animal Behaviour Science, 200*, 114-122. doi:10.1016/j.applanim.2017.12.007

Bakal-Weiss, M., Steinberg, D., Friedman, M. & Gati, I. (2010). Use of a sustained release chlorhexidine varnish as treatment of oral necrobacillosis in *Macropus* spp. *Journal of Zoo and Wildlife Medicine, 41*(2), 371-373.

Ballou, J. D., Lees, C., Faust, L. J., Long, S., Lynch, C., Bingaman Lackey, L. & Foose, T. J. (2010). Demographic and genetic management of captive populations. In D. G. Kleiman, K. Thompson, & C. Kirk-Baer (Eds.), *Wild mammals in captivity: Principles and techniques for zoo management* (2nd ed., pp. 219-252). Chicago, IL.: University of Chicago Press.

Bang, B. (1890). Om aarsagen til lokal nekrose. *Maamedsskr. F. Dyrlaeger, 2*, 235-259.

Barker, R. D. (1987). The diet of herbivores in the sheep rangelands. In G. Caughley, J. Short, & N. Shepherd (Eds.), *Kangaroos: Their ecology and management in the sheep rangelands of Australia* (pp. 69-83). Cambridge, England: Cambridge University Press.

Barker, S. (1971). The Dama wallaby in captivity. *International Zoo Yearbook, 11*(1), 17-20. doi:10.1111/j.1748-1090.1971.tb01829.x

Barker, S., Brown, D. G. & Calaby, J. H. (1963). Food regurgitation in the macropodidae. *Australian Journal of Biological Sciences, 25*, 430-432.

Barnes, T., Hinds, L., Jenkins, D., Coleman, G., Colebrook, A., Kyngdon, C., Gauci, C. & Lightowlers, M. (2009). Efficacy of the EG95 hydatid vaccine in a macropodid host, the tammar wallaby. *Parasitology, 136*(4), 461-468. doi:10.1017/S0031182009005526

Barrie, M. T. (2003, January). *Lumpy jaw in macropods.* Paper presented at the North American Veterinary Conference, Orlando, FL.

Bennett, G., Hickford, J., Sedcole, R. & Zhou, H. (2009). *Dichelobacter nodosus*, *Fusobacterium necrophorum* and the epidemiology of footrot. *Anaerobe, 15*(4), 173-176. doi:10.1016/j.anaerobe.2009.02.002

Bertelsen, M. F., Bojesen, A. M., Bisgaard, M., Petersen, A. & Christensen, H. (2012). *Pasteurella multocida* carriage in red-necked wallabies (*Macropus rufogriseus*). *Journal of Zoo and Wildlife Medicine, 43*(4), 726-729. doi:10.1638/2011-0051R.1

Beveridge, W. I. B. (1934). A study of twelve strains of *Bacillus necrophorus*, with observations on the oxygen intolerance of the organism. *The Journal of Pathology and Bacteriology, 38*(3), 467-491. doi:10.1002/path.1700380314

Biondi, M. & Zannino, L. G. (1997). Psychological stress, neuroimmunomodulation, and susceptibility to infectious diseases in animals and man: A review. *Psychotherapy and Psychosomatics, 66*(1), 3-26. doi:10.1159/000289101

Bird, P. S., Huynh, S. C., Davis, D., Love, D. N., Blackall, L. L. & Seymour, G. J. (2002). Oral disease in animals: The Australian perspective. Isolation and characterisation of black-pigmented bacteria from the oral cavity of marsupials. *Anaerobe, 8*(2), 79-87. doi:10.1006/anae.2002.0412

Blackett, T., McKenna, C., Kavanagh, L. & Morgan, D. (2017). The welfare of wild animals in zoological institutions: Are we meeting our duty of care? *International Zoo Yearbook, 51*(1), 187-202.

Blair, W. R. (1916). Report of the veterinarian of the zoological park. *New York Zoological Society Annual Report. 21st.* (pp. 77-81). New York, NY: New York Zoological Society.

Blair, W. R. (1924). Report of the veterinarian. *New York Zoological Society Annual Report. 29th.* (pp. 50-54). New York, NY: New York Zoological Society.

Blanden, D. R., Lewis, P. R. & Ferrier, G. R. (1987). Vaccination against lumpy jaw and measurement of antibody response in wallabies (*Macropus eugenii*). *Veterinary Record, 121*(3), 60-62. doi:10.1136/vr.121.3.60

Blecha, F. (2000). Immune system response to stress. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 111-121). Wallingford, England: CABI Publishing.

Blyde, D. (1993, October). *Common diseases and treatments in macropods.* Paper presented at the *Proceedings of the American Association of Zoo Veterinarians*, St Louis, Missouri, (pp. 168-170). Yulee, Florida: American Association of Zoo Veterinarians.

Blyde, D. (1994). Management and diseases of macropods. In *Proceedings. Wildlife: The TG Hungerford Refresher Course for Veterinarians* (Vol. 233, pp. 247-251). Dubbo, New South Wales: University of Sydney.

Blyde, D. (1999, September). *Advances in treating diseases in macropods.* Paper presented at the Wildlife in Australia: Healthcare and Management conference, Dubbo, New South Wales.

Bodley, K., McCracken, H. E. & Lynch, M. J. (2005). *Dental disease in macropod species at Melbourne Zoo* In C. Baer (Ed.), *Proceedings of the Joint Conference American Association of Zoo Veterinarians, American Association of Wildlife Veterinarians and Nutrition Advisory Group* (pp. 243-246). Omaha, NE: American Association of Zoo Veterinarians.

Boever, W. J. & Leathers, C. (1973). Pulmonary and encephalic infection secondary to lumpy jaw in kangaroos. *The Journal of Zoo Animal Medicine, 4*(4), 13-16. doi:10.2307/20094206

Bond, A. R. & Jones, D. N. (2014). Roads and macropods: Interactions and implications. *Australian Mammalogy, 36*(1), 1-14. doi:10.1071/AM13005

Booth, R. & Gage, L. J. (2008). Macropods. In L. J. Gage (Ed.), *Hand-rearing wild and domestic mammals* (pp. 63-74). Ames, Iowa: Blackwell Publishing Ltd.

Borland, D. (2006). *Studies on mortalities of eastern grey kangaroos (Macropus giganteus) at Serendip Wildlife Sanctuary.* Unpublished BSc Thesis, Univeristy of Melbourne, Melbourne, Victoria.

Borland, D., Coulson, G. & Beveridge, I. (2012). Oral necrobacillosis ('lumpy jaw') in a free-ranging population of eastern grey kangaroos (*Macropus giganteus*) in Victoria. *Australian Mammalogy, 34*(1), 29-35. doi:10.1071/AM10031

Boulton, K. A., Vogelnest, L. J. & Vogelnest, L. (2013). Dermatophytosis in zoo macropods: A questionnaire study. *Journal of Zoo and Wildlife Medicine, 44*(3), 555-563.

Boyd, A. G. C. (1929). Observations on diseases in captive wild animals with special reference to necrophorus infection in snakes and tortoises. *The Cornell Veterinarian, 19*, 33-39.

Braswell, L. (1991). Exotic animal dentistry. *The Compendium on continuing education for the practicing veterinarian (USA), 13*(8), 1229-1233.

Broadley, M. & Schweon, S. J. (2017). Get the facts about *Fusobacterium*. *Nursing, 47*(5), 64. doi:10.1097/01.NURSE.0000515524.23032.d5

Brookins, M. D., Rajeev, S., Thornhill, T. D., Kreinheder, K. & Miller, D. L. (2008). Mandibular and maxillary osteomyelitis and myositis in a captive herd of red kangaroos (*Macropus rufus*). *Journal of Veterinary Diagnostic Investigation, 20*(6), 846-849.

Broom, D. M. (2003). Transport stress in cattle and sheep with details of physiological, ethological and other indicators. *Deutsche Tierarztliche Wochenschrift, 110*(3), 83-88.

Broom, D. M. (2005). The effects of land transport on animal welfare. *Revue Scientifique Et Technique-Office International Des Epizooties, 24*(2), 683.

Buchanan-Smith, H. M. (2012). Mixed-species exhibition of Neotropical primates: Analysis of species combination success. *International Zoo Yearbook, 46*(1), 150-163.

Burbidge, A., Menkhorst, P., Ellis, M. & Copley, P. (2016). Western grey kangaroo. *Macropus fuliginosus*. Retrieved from The IUCN Red List of Threatened Species 2016: https://www.iucnredlist.org/species/40563/21953972

Burgdorf-Moisuk, A., Pye, G. W., Smith, J. A., Papendick, R., Ivy, J. A. & Hamlin-Andrus, C. (2012). A retrospective and prospective study of megaesophagus in the parma wallaby (*Macropus parma*) at the San Diego Zoo, California, USA. . *Journal of Zoo and Wildlife Medicine, 43*(2), 324-329.

Burton, J. D. (1981). *Studies into aspects of lumpy jaw in macropods.* Unpublished PhD thesis, University of Melbourne, Melbourne, Victoria.

Butler, R. (1981). Epidemiology and management of "lumpy jaw" in macropods. In M. Fowler (Ed.), *Wildlife diseases of the Pacific Basin and other countries.* (pp. 58-61). Sydney, New South Wales: Wildlife Disease Association.

Butler, R. & Burton, J. (1980). Necrobacillosis of macropods – control and therapy *Annual Proceedings of the American Association of Zoo Veterinarians* (pp. 137-140). Washington, D.C.: American Association of Zoo Veterinarians.

Cabana, F., Dierenfeld, E., Wirdateti, W., Donati, G. & Nekaris, K. (2017). Slow lorises (*Nycticebus* spp.) really are slow: A study of food passage rates. *International Journal of Primatology, 38*(5), 900-913. doi:10.1007/s10764-017-9986-5

Calaby, J. H. & Poole, W. E. (1971). Keeping kangaroos in captivity. *International Zoo Yearbook, 11*(1), 5-12. doi:10.1111/j.1748-1090.1971.tb01825.x

Campbell, R. D., Peralta, S., Fiani, N. & Scrivani, P. V. (2016). Comparing intraoral radiography and computed tomography for detecting radiographic signs of periodontitis and endodontic disease in dogs: An agreement study. *Frontiers in Veterinary Science, 3*(68). doi:10.3389/fvets.2016.00068

Canfield, P. J. & Cunningham, A. A. (1993). Disease and mortality in Australasian marsupials held at London Zoo, 1872-1972. *Journal of Zoo and Wildlife Medicine, 24*(2), 158-167. doi:10.2307/20095257

Caumul, R. & Polly, P. D. (2005). Phylogenetic and environmental components of morphological variation: skull, mandible, and molar shape in marmots (Marmota, Rodentia). *Evolution, 59*(11), 2460-2472.

Chen, M. M., Boardman, W. S., Smith, I., Goodman, A. E. & Brown, M. H. (2015). Characterisation of β-lactam resistance mediated by blaZ in staphylococci recovered from captive and free-ranging wallabies. *Journal of Global Antimicrobial Resistance, 3*(3), 184-189. doi:10.1016/j.jgar.2015.05.002

Chhour, K.-L., Hinds, L. A., Jacques, N. A. & Deane, E. M. (2010). An observational study of the microbiome of the maternal pouch and saliva of the tammar wallaby, *Macropus eugenii*, and of the gastrointestinal tract of the pouch young. *Microbiology, 156*(3), 798-808. doi:10.1099/mic.0.031997-0

Chirino-Trejo, M., Woodbury, M. R. & Huang, F. (2003). Antibiotic sensitivity and biochemical characterization of *Fusobacterium* spp. and *Arcanobacterium pyogenes* isolated from farmed white-tailed deer (*Odocoileus virginianus*) with necrobacillosis. *Journal of Zoo and Wildlife Medicine, 34*(3), 262-268. doi:10.1043/1042-7260(2003)034(0262:ASABCO)2.0.CO;2

Choudhary, S., Bhanuprakash, A. & Mahendran, K. (2016). Therapeutic management of actinomycosis in a calf. *Intas Polivet, 17*(2), 578-580.

Christensen, H. B. (2014). Similar associations of tooth microwear and morphology indicate similar diet across marsupial and placental mammals. *PloS One, 9*(8). doi:10.1371/journal.pone.0102789

Christley, R. M. (2016). Questionnaire survey response rates in equine research. *Equine Veterinary Journal, 48*(2), 138-139. doi:10.1111/evj.12552

Clancy, T. & Croft, D. (1989). Space-use patterns of the common wallaroo *Macropus robustus erubescens* in the arid zone. In G. Grigg, P. Jarman, & I. Hume (Eds.), *Kangaroos, wallabies and rat-kangaroos* (Vol. 2, pp. 603-609). Sydney, New South Wales: Surrey Beatty and Sons.

Clancy, T. F. & Croft, D. B. (1991). Differences in habitat use and grouping behavior between macropods and eutherian herbivores. *Journal of Mammalogy, 72*(3), 441-449.

Clarke, D. E. (2003). Oral biology and disorders of chiroptera, insectivores, monotremes, and marsupials. *Veterinary Clinics of North America: Exotic Animal Practice, 6*(3), 523-564. doi:10.1016/S1094-9194(03)00033-1

Clauss, M., Franz-Odendaal, T. A., Brasch, J., Castell, J. C. & Kaiser, T. (2007). Tooth wear in captive giraffes (*Giraffa camelopardalis*): mesowear analysis classifies free-ranging specimens as browsers but captive ones as grazers. *Journal of Zoo and Wildlife Medicine, 38*(3), 433-445. doi:10.1638/06-032.1

Clauss, M., Kaiser, T. & Hummel, J. (2008). The morphophysiological adaptations of browsing and grazing mammals *The ecology of browsing and grazing* (pp. 47-88). Berlin, Germany: Springer.

Cockburn, A. (1997). Living slow and dying young: Senescence in marsupials. In N. Saunders & L. A. Hinds (Eds.), *Marsupial biology: recent research, new perspectives* (pp. 163-171). Sydney, New South Wales: University of New South Wales Press.

Coe, J. C. (2003). Steering the ark toward Eden: design for animal well-being. *Journal of the American Veterinary Medical Association, 223*(7), 977-980.

Coulson, G. (1997). Repertoires of social behaviour in captive and free-ranging grey kangaroos, *Macropus giganteus* and *Macropus fuliginosus* (Marsupialia: Macropodidae). *Journal of Zoology, 242*(1), 119-130.

Cox-Witton, K., Reiss, A., Woods, R., Grillo, V., Baker, R. T., Blyde, D. J., Boardman, W., Cutter, S., Lacasse, C., McCracken, H., Pyne, M., Smith, I., Vitali, S., Vogelnest, L., Wedd, D., Phillips, M., Bunn, C. & Post, L. (2014). Emerging infectious diseases in free-ranging wildlife – Australian zoo based wildlife hospitals contribute to national surveillance. *PloS One, 9*(5), e95127. doi:10.1371/journal.pone.0095127

Craig, L. E., Dittmer, K. E. & Thompson, K. G. (2016). Bones and joints. In G. Maxie (Ed.), *Jubb, Kennedy & Palmer's pathology of domestic animals: Volume 1* (6th ed., pp. 16-163). St. Louis, MO: Elsevier.

Crandall, L. S. (1964). *The management of wild mammals in captivity*. Chicago, Illinois: University of Chicago Press.

Crossley, D. A. & Aiken, S. (2004). Small mammal dentistry. Part II: Surgical treatment of dental abscesses in rabbits. In K. E. Quesenberry & S. Carpenter (Eds.), *Ferrets, rabbits and rodents: Clinical medicine and surgery* (2nd ed., pp. 370-382). St. Louis, Missouri: Saunders.

Da Silva, M. A. O., Kortegaard, H. E., Choong, S. S., Arnbjerg, J. & Bertelsen, M. F. (2011). Resorptive tooth root lesions in the Malayan tapir (*Tapirus indicus*). *Journal of Zoo and Wildlife Medicine, 42*(1), 40-43.

Damuth, J. & Janis, C. M. (2013). A comparison of observed molar wear rates in extant herbivorous mammals. *Annales Zoologici Fennici, 51*, 188-200.

Dawson, T. (1995). *Kangaroos: Biology of the largest marsupials*. Sydney, New South Wales: University of New South Wales Press.

Dawson, T. J. (2012). *Kangaroos* (2nd ed.). Melbourne, Victoria: CSIRO Publishing.

Defra. (2011). *Foot and mouth disease control strategy for Great Britain*. London, United Kingdom: Department for Environment, Food and Rural Affairs.

Dembiec, D. P., Snider, R. J. & Zanella, A. J. (2004). The effects of transport stress on tiger physiology and behavior. *Zoo Biology, 23*(4), 335-346. doi:10.1002/zoo.20012

Dent, V. E. (1979). The bacteriology of dental plaque from a variety of zoo-maintained mammalian species. *Archives of Oral Biology, 24*(4), 277-282.

Department of the Environment and Heritage (1990). Code of practice for the humane shooting of kangaroos. (2nd ed.). Canberra, Australian Capital Territory: Environment Australia.

Dickins, A., Clark, C. C., Kaler, J., Ferguson, E., O'Kane, H. & Green, L. E. (2016). Factors associated with the presence and prevalence of contagious ovine digital dermatitis: A 2013 study of 1136 random English sheep flocks. *Preventive Veterinary Medicine, 130*, 86-93. doi:10.1016/j.prevetmed.2016.06.009

Divers, S. J. & Lawton, M. P. C. (1999). *Antibiotic-impregnated polymethylmethacrylate beads as a treatment for osteomyelitis in reptiles*. Paper presented at the Proceedings of the Association of Reptilian and Amphibian Veterinarians, Columbus, OH, 5-9 October (pp. 145-147). Gainesville, FL: Association of Reptilian and Amphibian Veterinarians.

Dohms, J. E. & Metz, A. (1991). Stress - mechanisms of immunosuppression. *Veterinary Immunology and Immunopathology, 30*(1), 89-109. doi:10.1016/0165-2427(91)90011-Z

Domínguez-Roldan, R., Pérez-Martínez, M., Rosetti, M., Arias-Hernández, D., Bernal-Fernández, G., Flores-Pérez, F. & Hallal-Calleros, C. (2018). High frequency of *Taenia pisiformis* metacestodes and high sex-associated susceptibility to cysticercosis in naturally infected wild rabbits. *Parasitology Research, 117*(7), 2201-2206.

Dorman, N. & Bourne, D. (2010). Canids and ursids in mixed-species exhibits. *International Zoo Yearbook, 44*(1), 75-86. doi:10.1111/j.1748-1090.2009.00108.x

Dudzinski, M., Newsome, A., Merchant, J. & Bolton, B. (1977). Comparing the two usual methods for aging Macropodidae on tooth-classes in the agile wallaby. *Wildlife Research, 4*(3), 219-221. doi:10.1071/WR9770219

Emery, D. L., Vaughan, J. A., Clark, B. L., Dufty, J. H. & Stewart, D. J. (1985). Cultural characteristics and virulence of strains of *Fusobacterium necrophorum* isolated from the feet of cattle and sheep. *Australian Veterinary Journal, 62*(2), 43-46.

Eriksen, E. (1964). Observations on diseases in kangaroos in Copenhagen Zoo. *Tijdschrift voor Diergeneeskunde, 89*(suppl. 1), 126-137.

Fagan, D. A., Oosterhuis, J. E. & Benirschke, K. (2005). 'Lumpy jaw' in exotic hoof stock: A histopathologic interpretation with a treatment proposal. *Journal of Zoo and Wildlife Medicine, 36*(1), 36-43. doi:10.1605/01.301-0000384089.2006

Fagan, D. A., Oosterhuis, J. E. & Fagan Kirkman, J. E. (1998). A review of the expanding field of exotic animal oral health care - Veterinary dentistry. *Journal of Veterinary Dentistry, 15*(3), 117-128. doi:10.1177/089875649801500301

Fèvre, E. M., Bronsvoort, B. M. D. C., Hamilton, K. A. & Cleaveland, S. (2006). Animal movements and the spread of infectious diseases. *Trends in Microbiology, 14*(3), 125-131. doi:10.1016/j.tim.2006.01.004

Fiani, N. (2015). Dental radiology. In L. Vogelnest & G. Allan (Eds.), *Radiology of Australian mammals* (pp. 205-222). Clayton, Victoria: CSIRO Publishing.

Finnie, E. P. (1976). Necrobacillosis in kangaroos. In L. A. Page (Ed.), *Wildlife diseases* (pp. 511-518). Boston, MA: Springer.

Firestone, S. M., Schemann, K. A., Toribio, J.-A. L. M. L., Ward, M. P. & Dhand, N. K. (2011). A case-control study of risk factors for equine influenza spread onto horse premises during the 2007 epidemic in Australia. *Preventive Veterinary Medicine, 100*(1), 53-63. doi:10.1016/j.prevetmed.2011.03.002

Fisher, D. O., Owens, I. P. & Johnson, C. N. (2001). The ecological basis of life history variation in marsupials. *Ecology, 82*(12), 3531-3540.

Fitch, H. M. & Fagan, D. A. (1982). Focal palatine erosion associated with dental malocclusion in captive cheetahs. *Zoo Biology, 1*(4), 295-310.

Flannery, T. (1995). *Mammals of New Guinea* (2nd ed.). Sydney, New South Wales: Reed Books.

Flannery, T. F., Martin, R. & Szalay, A. (1996). *Tree kangaroos: A curious natural history*. Port Melbourne, Victoria: Reed Books.

Fleming, M. & Burn, C. C. (2014). Behavioural assessment of dental pain in captive Malayan sun bears (*Helarctos malayanus*). *Animal Welfare, 23*(2), 131-140. doi:10.1016/j.applanim.2006.05.032

Föllmi, J., Steiger, A., Walzer, C., Robert, N., Geissbühler, U., Doherr, M. G. & Wenker, C. (2007). A scoring system to evaluate physical condition and quality of life in geriatric zoo mammals. *Animal Welfare, 16*(3), 309-318.

Fox, H. (1923). *Disease in captive wild mammals and birds: Incidence, description, comparison*. Philadelphia, PA: J.B. Lippincott.

Frick, W. F., Puechmaille, S. J. & Willis, C. K. (2016). White-nose syndrome in bats. In C. C. Voigt & T. Kingston (Eds.), *Bats in the Anthropocene: Conservation of bats in a changing world* (pp. 245-262). Cham, Switzerland: Springer.

Gamble, K. C. (2004). Marsupial care and husbandry. *Veterinary Clinics of North America: Exotic Animal Practice, 7*(2), 283-298. doi:10.1016/j.cvex.2004.01.002

Gansloßer, U. (1989). Agonistic behaviour in macropodoids: A review. In G. Grigg, P. Jarman, & I. Hume (Eds.), *Kangaroos, wallabies and rat-kangaroos* (Vol. 2, pp. 475-503). Sydney, New South Wales: Surrey Beatty and Sons.

Gansloßer, U. (1995). The importance of age/sex class relationships for captive-breeding: A review of Macropodoidea (kangaroos, wallabies and rat-kangaroos) as a case study. *Animal Welfare, 4*(3), 239-249.

Garnick, S., Di Stefano, J., Moore, B. D., Davis, N. E., Elgar, M. A. & Coulson, G. (2018). Interspecific and intraspecific relationships between body mass and diet quality in a macropodid community. *Journal of Mammalogy, 99*(2), 428-439. doi:10.1093/jmammal/gyx183

Gillett, A. & Hanger, J. (2019). Koala. In L. Vogelnest & T. Portas (Eds.), *Current therapy in medicine of Australian mammals* (pp. 463-486). Clayton South, Victoria: CSIRO Publishing.

Glatt, S. E., Francl, K. E. & Scheels, J. L. (2008). A survey of current dental problems and treatments of zoo animals. *International Zoo Yearbook, 42*(1), 206-213. doi:10.1111/j.1748-1090.2007.00032.x

Grandin, T. (1997). Assessment of Stress during Handling and Transport. *Journal of Animal Science, 75*(1), 249-257. doi:10.2527/1997.751249x

Gray, J. E. (1821). On the natural arrangement of vertebrose animals. *London Medical Repository, 15*(1), 296-310.

Green-Barber, J. M., Stannard, H. J. & Old, J. M. (2018). A suspected case of myopathy in a free-ranging eastern grey kangaroo (*Macropus giganteus*). *Australian Mammalogy, 40*(1), 122-126. doi:10.1071/AM16054

Gregory, N. G. (2004). *Physiology and behaviour of animal suffering*. Oxford, United Kingdom: Blackwell Science Ltd.

Griffith, J. E., Dhand, N. K., Krockenberger, M. B. & Higgins, D. P. (2013). A retrospective study of admission trends of koalas to a rehabilitation facility over 30 years. *Journal of Wildlife Diseases, 49*(1), 18-28. doi:10.7589/2012-05-135

Grífols, J., Perpiñán, D. & ECZM, L. M. D. (2013). Resolution of lumpy jaw in a red kangaroo (*Macropus rufus*) by the combination of dental extraction and use of vancomycin-methylmethacrylate beads. *In: Proceedings of the 1st International Conference on Avian, Herpetological and Exotic mammal Medicine* (pp. 337-339). 20-26 April, Wiesbaden, Germany.

Gulland, F., Lewis, J., Kock, R. A. & Kirkwood, J. K. (1987). Vaccination against lumpy jaw in wallabies. *Veterinary Record, 121*, 311.

Hahn, G. & Becker, B. (1984). Assessing livestock stress. *Agricultural Engineering (USA), 65*, 15-17.

Harcourt-Brown, F. M. (1995). A review of clinical conditions in pet rabbits associated with their teeth. *Veterinary Record, 137*(14), 341-346.

Hartley, M. P. & Sanderson, S. (2003). Use of antibiotic impregnated polymethylmethacrylate beads for the treatment of chronic mandibular osteomyelitis in a Bennett's wallaby (*Macropus rufogriseus rufogriseus*). *Australian Veterinary Journal, 81*(12), 742-744. doi:10.1111/j.1751-0813.2003.tb14604.x

Hartstone-Rose, A., Selvey, H., Villari, J. R., Atwell, M. & Schmidt, T. (2014). The three-dimensional morphological effects of captivity. *PloS One, 9*(11), e113437. doi:10.1371/journal.pone.0113437

Hartup, B. K., Dhondt, A. A., Sydenstricker, K. V., Hochachka, W. M. & Kollias, G. V. (2001). Host range and dynamics of mycoplasmal conjunctivitis among birds in North America. *Journal of Wildlife Diseases, 37*(1), 72-81.

Hawkey, C. M., Ashton, D. G., Hart, M. G., Cindery, R. N. & Jones, D. M. (1982). Haematological findings in red-necked wallabies (*Protemnodon rufogrisea*) with necrobacillosis. *Research in Veterinary Science, 33*(3), 290-294.

Herter, U., Schneider, I. & Gansloßer, U. (1985). Some remarks on the behavioural repertoire of the parma walllaby, *Macropus parma* Waterhouse. *Zoologischer Anzeiger, 215*(5-6), 287-297.

Hill, S. P. & Broom, D. M. (2009). Measuring zoo animal welfare: Theory and practice. *Zoo Biology, 28*(6), 531-544. doi:10.1002/zoo.20276

Hillmann, E., Mayer, C. & Schrader, L. (2004). Lying behaviour and adrenocortical response as indicators of the thermal tolerance of pigs of different weights. *Animal Welfare, 13*(3), 329-335.

Hing, S., Narayan, E., Thompson, R. & Godfrey, S. (2017). Identifying factors that influence stress physiology of the woylie, a critically endangered marsupial. *Journal of Zoology, 302*(1), 49-56. doi:10.1111/jzo.12428

Hing, S., Narayan, E., Thompson, R. C. A. & Godfrey, S. (2014). A review of factors influencing the stress response in Australian marsupials. *Conservation Physiology, 2*(1), cou027. doi:10.1093/conphys/cou027

Hoefs, M. & Bunch, T. D. (2001). Lumpy jaw in wild sheep and its evolutionary implications. *Journal of Wildlife Diseases, 37*(1), 39-48. doi:10.7589/0090-3558-37.1.39

Höhn, M., Kronschnabl, M. & Gansloßer, U. (2000). Similarities and differences in activities and agonistic behavior of male Eastern grey kangaroos (*Macropus giganteus*) in captivity and the wild. *Zoo Biology, 19*(6), 529-539. doi:10.1002/1098-2361(2000)19:6<529::AID-ZOO5>3.0.CO;2-L

Hollister, N. (1917). Some effects of environment and habit on captive lions. . *Proceedings of the United States National Museum, 53*(2196), 177-193.

Horton, D. & Samuel, J. (1978). Paleopathology of a fossil macropod population. *Australian Journal of Zoology, 26*(2), 279-292. doi:10.1071/ZO9780279

Hosey, G. & Melfi, V. (2015). Are we ignoring neutral and negative human–animal relationships in zoos? *Zoo Biology, 34*(1), 1-8. doi:10.1002/zoo.21182

Hosey, G. R. (2000). Zoo animals and their human audiences: What is the visitor effect? *Animal Welfare, 9*(4), 343-357.

Hosey, G. R., Melfi, V. & Pankhurst, S. (2013). *Zoo animals: behaviour, management and welfare* (2nd ed.). Oxford, United Kingdom: Oxford University Press.

Huber, I., Herceg, M. & Maran, B. (1976). Zu den erkrankungen der Australischen tiere im Zoologischen Garten der Stadt Zagreb. *VISZ, 18*, 13-16.

Hume, I., Jarman, P., Renfree, M., Temple-Smith, P. D., Walton, D. & Richardson, B. (1989). Macropodidae. In D. Walton & B. Richardson (Eds.), *Fauna of Australia: Vol 1B, Mammalia* (pp. 679-715). Canberra: Australian Government Publishing Service.

Hume, I. D. (1982). *Digestive physiology and nutrition of marsupials*. Cambridge, United Kingdom: Cambridge University Press.

International Union for Conservation of Nature. (2016). *The IUCN Red List of Threatened Species*. Version 2016-2. Retrieved from http://www.iucnredlist.org/

Irwin, D. H. G. & Cameron, C. (1962). A note on oral infection in the kangaroo (*Macropus rufus*) and wallaby (*Macropus ruficollis*)(Desmarest). *Journal of the South African Veterinary Medical Association, 33*(2), 231-232.

Jackson, S. (2003). *Australian mammals: Biology and captive management*. Collingwood, Victoria: CSIRO Publishing.

Jarman, P. J. (1984). The dietary ecology of macropod marsupials *Proceedings of the Nutrition Society of Australia* (Vol. 9, pp. 82-87). n.p.: The Society.

Jensen, C. O. (1913). Die vom nekrosebacillus (*Bacillus necroseos*) hervorgerufenen krankheiten. In W. Kolle & A. Wasserman (Eds.), *Handbuch der pathogenen mikroorganismen* (2nd ed., Vol. 6, pp. 234-250). Jena, Germany: Fischer Verkag.

Johnson-Delaney, C. A. (2014). Captive marsupial nutrition. *Veterinary Clinics of North America: Exotic Animal Practice, 17*(3), 415-447. doi:10.1016/j.cvex.2014.05.006

Kaiser, T. M., Brasch, J., Castell, J. C., Schulz, E. & Clauss, M. (2009). Tooth wear in captive wild ruminant species differs from that of free-ranging conspecifics. *Mammalian Biology - Zeitschrift für Säugetierkunde, 74*(6), 425-437. doi:10.1016/j.mambio.2008.09.003

Kamiya, Y., Timonen, V., Romero-Ortuno, R. & Kenny, R. (2013). Back to basics or into a brave new world? The potential and pitfalls of biomarkers in explaining the pathways between social engagement and health. In M. N. Hoque, M. A. McGehee, & B. S. Bradshaw (Eds.), *Applied demography and public health* (Vol. 3, pp. 313 - 336). New York, NY: Springer.

Kane, L. P., Langan, J. N., Adkesson, M. J., Chinnadurai, S. K., Nevitt, B. N. & Drees, R. (2017). Treatment of mandibular osteomyelitis in two red-necked wallabies (*Macropus rufogriseus*) by means of intensive long-term parenteral drug administration and serial computed tomographic monitoring. *Journal of the American Veterinary Medical Association, 251*(9), 1070-1077. doi:10.2460/javma.251.9.1070

Kaufmann, J. H. (1974). The ecology and evolution of social organization in the kangaroo family (Macropodidae). *American Zoologist, 14*(1), 51-62.

Keane, C., Taylor, M. R. H., Wilson, P., Smith, L., Cunningham, B., Devine, P. & English, L. F. (1977). *Bacteroides ruminicola* as a possible cause of “lumpy-jaw” in Bennett's wallabies. *Veterinary Microbiology, 2*(2), 179-183. doi:10.1016/0378-1135(77)90007-4

Kenny, D., Cambre, R. C., Lewandowski, A., Pelto, J. A., Irlbeck, N. A., Wilson, H., Mierau, G. W., Sill, M. F. G. & Garcia, M. A. P. (1993). Suspected vitamin D_3 toxicity in pacas (*Cuniculus paca*) and agoutis (*Dasyprocta aguti*). *Journal of Zoo and Wildlife Medicine, 24*(2), 129-139.

Ketz, V. (1997, May). *Necrobacillosis in zoo kangaroos. Hypotheses of impact of management conditions.* Paper presented at the Erkrankungen der Zootiere. Verhandlungsbericht des 38 Internationalen Symposiums über die Erkrankungen der Zoo und Wildiere, Zurich, Switzerland.

Kido, N., Chikuan, A., Omiya, T., Dan, M. & Nomura, M. (2013). Retrospective study of oral necrobacillosis in 54 swamp wallabies. *Veterinary Record, 173*(5), 118. doi:10.1136/vr.101694

Kido, N., Tanaka, S., Wada, Y., Sato, S. & Omiya, T. (2018). Molar eruption and identification of the eastern grey kangaroo (*Macropus giganteus*) at different ages. *Journal of Veterinary Medical Science, 80*(4), 648-652. doi:10.1292/jvms.17-0069

Kilgallon, C. P., Bicknese, B. & Fagan, D. A. (2010). Successful treatment of chronic periapical osteomyelitis in a parma wallaby (*Macropus parma*) using comprehensive endodontic therapy with apicoectomy. *Journal of Zoo and Wildlife Medicine, 41*(4), 703-709.

King, J. & Bradshaw, S. (2010). Stress in an island kangaroo? The Barrow Island euro, *Macropus robustus isabellinus*. *General and Comparative Endocrinology, 167*(1), 60-67. doi:10.1016/j.ygcen.2010.02.018

Kirkpatrick, T. (1964). Molar progression and macropod age. *Queensland Journal of Agricultural and Animal Sciences, 21*, 163-165.

Kirkpatrick, T. (1965). Studies of Macropodidae in Queensland. 2. Age estimation in the grey kangaroo, the red kangaroo, the eastern wallaroo and the red-necked wallaby, with notes on dental abnormalities. *Queensland Journal of Agricultural and Animal Sciences, 22*, 301-317.

Kirkpatrick, T. (1970). Studies of the Macropodidae in Queensland. 8. Age estimation in the red kangaroo (*Megaleia rufa* (Desmarest)). *Queensland Journal of Agricultural and Animal Sciences, 27*, 461-462.

Kirkwood, J. K., Gulland, F. M. D., Needham, J. R. & Vogler, M. G. (1988). Pharmacokinetics of oxytetracycline in clinical cases in the red-necked wallaby (*Macropus rufogriseus*). *Research in Veterinary Science, 44*(3), 335-337. doi:10.1016/S0034-5288(18)30867-1

Kottek, M., Grieser, J., Beck, C., Rudolf, B. & Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift, 15*(3), 259-263. doi:10.1127/0941-2948/2006/0130

Kronberger, H. & Schüppel, K. F. (1976). *Todesursachen von Kanguruhs - Durchsicht der Secktionspotokolle von 1853 bis 1975.* Paper presented at the Erkrankungen der Zootiere. Verhandlungsbericht des XVIII Internationalen Symposiums über die Erkankungen der Zootiere, Innsbruck, Austria (pp. 27-30). Berlin, Germany: Akadamie-Jerlag.

Laule, G. E., Bloomsmith, M. A. & Schapiro, S. J. (2003). The use of positive reinforcement training techniques to enhance the care, management, and welfare of primates in the laboratory. *Journal of Applied Animal Welfare Science, 6*(3), 163-173. doi:10.1207/S15327604JAWS0603_02

Le Souef, A. S. & Seddon, H. R. (1929). Streptothrix disease in kangaroos. *Australian Veterinary Journal, 5*(2), 79-80. doi:10.1111/j.1751-0813.1929.tb00708.x

Learmonth, M. J., Sherwen, S. & Hemsworth, P. H. (2018). The effects of zoo visitors on Quokka (*Setonix brachyurus*) avoidance behavior in a walk-through exhibit. *Zoo Biology, 37*(4), 223-228. doi:10.1002/zoo.21433

Leary, T., Seri, L., Wright, D., Hamilton, S., Helgen, K., Singadan, R., Menzies, J., Allison, A., James, R., Dickman, C., Aplin, K., Flannery, T., Martin, R. & Salas, L. (2016). Goodfellow's tree kangaroo. *Dendrolagus goodfellowi.* The IUCN Red List of Threatened Species 2016. Retrieved from https://www.iucnredlist.org/species/6429/21957524 doi:10.2305/IUCN.UK.2016-2.RLTS.T6429A21957524.en.

Lee, A. & Ward, S. (1989). Life histories of macropodoid marsupials. In G. Grigg, P. Jarman, & I. Hume (Eds.), *Kangaroos, wallabies and rat-kangaroos* (Vol. 1, pp. 105-115). Sydney, New South Wales: Surrey Beatty and Sons.

Lee, K. J., Sasaki, M., Miyauchi, A., Kishimoto, M., Shimizu, J., Iwasaki, T., Miyake, Y.-I. & Yamada, K. (2011). Virtopsy in a red kangaroo with oral osteomyelitis. *Journal of Zoo and Wildlife Medicine, 42*(1), 128-130. doi:10.1638/2010-0015.1

Lentle, R. G., Hume, I. D., Stafford, K. J., Kennedy, M., Haslett, S. & Springett, B. P. (2003). Molar progression and tooth wear in tammar (*Macropus eugenii*) and parma (*Macropus parma*) wallabies. *Australian Journal of Zoology, 51*(2), 137-151. doi:10.1071/ZO02008

Leus, K., Traylor-Holzer, K. & Lacy, R. C. (2011). Genetic and demographic population management in zoos and aquariums: Recent developments, future challenges and opportunities for scientific research. *International Zoo Yearbook, 45*(1), 213-225. doi:10.1111/j.1748-1090.2011.00138.x

Lewis, J. C. M., Fitzgerald, A. J., Gulland, F. M. D., Hawkey, C. M., Kertesz, P., Kirkwood, J. K. & Kock, R. A. (1989). Observations on the treatment of necrobacillosis in wallabies. *British Veterinary Journal, 145*(4), 394-396. doi:10.1016/0007-1935(89)90040-7

Lewis, S. C., Karoly, D. J., King, A. D., Perkins, S. E. & Donat, M. G. (2017). Mechanisms explaining recent changes in Australian climate extremes. In S. Y. S. Wang, J. H. Yoon, Funk, C.C., & R. R. Gillies (Eds.), *Climate Extremes: Patterns and Mechanisms* (pp. 249-263). Hoboken, NJ: John Wiley & Sons, Inc.

Lindau, K. H. (1964). Über todesfälle von kängurus im Zoologischen Garten Köln. *Tijdschrift voor Diergeneeskunde, 89*(Suppl. 1), 143-148.

Littleton, J. T. & Durizch Littleton, M. L. (1996). Conventional tomography. In R. A. Gagliardi, B. L. McClennan, & N. Knight (Eds.), *A history of the radiological sciences* (Vol. 3, pp. 368-401). Reston, VA: Radiology Centennial, Inc.

MacDonald, K. & Labisky, R. F. (2004). Lumpy jaw in white-tailed deer subjected to a severe flood in the Florida Everglades. *Florida Scientist, 67*(1), 43-47.

MacFarlane, A. & Coulson, G. (2005). Sexual segregation in Australian marsupials. In K. E. Ruckstuhl & P. Neuhaus (Eds.), *Sexual segregation in vertebrates: Ecology of the two sexes* (pp. 254–279). Cambridge, United Kingdom: Cambridge University Press.

Marai, I. F. M. & Rashwan, A. (2004). Rabbits behavioural response to climatic and managerial conditions: A review. *Archives Animal Breeding, 47*(5), 469-482.

Marshall, A. R., Deere, N. J., Little, H. A., Snipp, R., Goulder, J. & Mayer-Clarke, S. (2016). Husbandry and enclosure influences on penguin behavior and conservation breeding. *Zoo Biology, 35*(5), 385-397. doi:10.1002/zoo.21313

Martin, L. B. (2009). Stress and immunity in wild vertebrates: Timing is everything. *General and Comparative Endocrinology, 163*(1), 70-76. doi:10.1016/j.ygcen.2009.03.008

Masand, A., Kumar, N. & Patial, V. (2015). Actinomycosis (lumpy jaw) in cow: A case report. *Comparative Clinical Pathology, 24*(3), 541-543. doi:10.1007/s00580-014-1939-1

Mason, G. J. (2010). Species differences in responses to captivity: stress, welfare and the comparative method. *Trends in Ecology & Evolution, 25*(12), 713-721. doi:10.1016/j.tree.2010.08.011

Mathews, F., Moro, D., Strachan, R., Gelling, M. & Buller, N. (2006). Health surveillance in wildlife reintroductions. *Biological Conservation, 131*(2), 338-347. doi:10.1016/j.biocon.2006.04.011

Mayberry, C. (2009). *Management of reproduction in western grey kangaroos (Macropus fuliginosus).* Unpublished PhD thesis, University of Western Australia, Perth, Western Australia.

Mayberry, C., Bencini, R., Mawson, P. & Maloney, S. (2018). Estimating the age of western grey kangaroos (*Macropus fuliginosus ocydromus*). *Australian Mammalogy, 40*(2), 254-261. doi:10.1071/AM16058

McArthur, C. & Sanson, G. D. (1988). Tooth wear in eastern grey kangaroos (*Macropus giganteus*) and western grey kangaroos (*Macropus fuliginosus*), and its potential influence on diet selection, digestion and population parameters. *Journal of Zoology, 215*(3), 491-504. doi:10.1111/j.1469-7998.1988.tb02855.x

McKenzie, S. & Deane, E. (2005). Faecal corticosteroid levels as an indicator of well-being in the tammar wallaby, *Macropus eugenii*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 140*(1), 81-87. doi:10.1016/j.cbpb.2004.11.002

McLelland, D. J. (2019). Macropod progressive periodontal disease ('lumpy jaw'). In L. Vogelnest & T. Portas (Eds.), *Current therapy in medicine of Australian mammals* (pp. 451-462). Clayton South, Victoria: CSIRO Publishing.

McLelland, D. J., Barker, I. K., Crawshaw, G., Hinds, L. A., Spilsbury, L. & Johnson, R. (2011). Single-dose pharmacokinetics of oxytetracycline and penicillin G in tammar wallabies (*Macropus eugenii*). *Journal of Veterinary Pharmacology and Therapeutics, 34*(2), 160-167. doi:10.1111/j.1365-2885.2010.01216.x

McLelland, D. J., Rich, B. G. & Holz, P. H. (2009). The pharmacokinetics of single dose intramuscular amoxicillin trihydrate in tammar wallabies (*Macropus eugenii*). *Journal of Zoo and Wildlife Medicine, 40*(1), 113-116. doi:10.1638/2008-0104.1

McLeod, S. R., Druhan, J. P. & Hacker, R. B. (2006). Estimating the age of kangaroos using eye lens weight. *Wildlife Research, 33*(1), 25-28. doi:10.1071/WR04077

McMahon, C., Wiggins, N., French, V., McCallum, H. & Bowman, D. (2013). A report of capture myopathy in the Tasmanian pademelon (*Thylogale billardierii*). *Animal Welfare, 22*(1), 1-4. doi:10.7120/09627286.22.1.001

Meadows, G. W. (1981). *Some aspects of the management and diseases of captive wallabies at the Auckland Zoo.* Paper presented at the Proceedings of the First Symposium on Marsupials in New Zealand, Auckland, New Zealand. Auckland, New Zealand: Auckland Zoo, New Zealand.

Meehan, C. L., Mench, J. A., Carlstead, K. & Hogan, J. N. (2016). Determining connections between the daily lives of zoo elephants and their welfare: An epidemiological approach. *PloS One, 11*(7), e0158124. doi:10.1371/journal.pone.0158124

Melbourne Zoo. (2007). *Computed tomography report - kangaroo*. Melbourne Zoo. Melbourne, Victoria.

Melfi, V. A. (2009). There are big gaps in our knowledge, and thus approach, to zoo animal welfare: A case for evidence-based zoo animal management. *Zoo Biology, 28*(6), 574-588. doi:10.1002/zoo.20288

Mellen, J. D. (1994). Survey and interzoo studies used to address husbandry problems in some zoo vertebrates. *Zoo Biology, 13*(5), 459-470. doi:10.1002/zoo.1430130508

Miller, A. (2001). *Parma wallaby (Macropus parma): Resource manual*. Providence, RI: Roger Williams Park Zoo.

Miller, W. & Beighton, D. (1979). Bone abnormalities in two groups of macropod skulls: A clue to the origin of lumpy jaw. *Australian Journal of Zoology, 27*(5), 681-689. doi:10.1071/ZO9790681

Miller, W. A., Beighton, D. & Butler, R. (1978). *Histological and osteological observations on the early stages of lumpy jaw.* Paper presented at the Comparative Pathology of Zoo Animals: Proceedings of a Symposium held at the National Zoological Park, Washington, DC, (pp. 231-239). Washington, DC: Smithsonian Institute.

Mitchell, D. R., Sherratt, E., Ledogar, J. A. & Wroe, S. (2018). The biomechanics of foraging determines face length among kangaroos and their relatives. *Proceedings of the Royal Society B: Biological Sciences, 285*(1881), 20180845. doi:10.1098/rspb.2018.0845

Moberg, G. P. (1985). Influence of stress on reproduction: Measure of well-being. In G. P. Moberg (Ed.), *Animal stress* (pp. 245-267). New York, NY: Springer.

Moberg, G. P. (2000). Biological response to stress: Implications for animal welfare. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 1-22). Wallingford, United Kingdom: CABI Publishing.

Morgan, K. N. & Tromborg, C. T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science, 102*(3–4), 262-302. doi:10.1016/j.applanim.2006.05.032

Mouquet, A. (1923). Maladie de Schmorl chez un kangourou. *Bulletin de la Société Centre de Médecine Vétérinaire, 76*, 419-423.

Munday, B. (1978). *Marsupial disease.* Paper presented at the Fauna: Proceedings No. 36 of a Course for Veterinarians (pp. 335-386). Sydney, New South Wales: Post-Graduate Committee for Veterinary Science, University of Sydney.

Munn, A. J., Skeers, P., Kalkman, L., McLeod, S. R. & Dawson, T. J. (2014). Water use and feeding patterns of the marsupial western grey kangaroo (*Macropus fuliginosus melanops*) grazing at the edge of its range in arid Australia, as compared with the dominant local livestock, the Merino sheep (*Ovis aries*). *Mammalian Biology - Zeitschrift für Säugetierkunde, 79*(1), 1-8. doi:10.1016/j.mambio.2013.03.003

Mysterud, A. (2000). The relationship between ecological segregation and sexual body size dimorphism in large herbivores. *Oecologia, 124*(1), 40-54. doi:10.1007/s004420050023

Nagaraja, T., Narayanan, S., Stewart, G. & Chengappa, M. (2005). *Fusobacterium necrophorum* infections in animals: pathogenesis and pathogenic mechanisms. *Anaerobe, 11*(4), 239-246. doi:10.1016/j.anaerobe.2005.01.007

Narayan, E. (2019). Physiological stress levels in wild koala sub-populations facing anthropogenic induced environmental trauma and disease. *Scientific Reports, 9*(1), 6031. doi:10.1038/s41598-019-42448-8

Narayan, E. J. & Hero, J.-M. (2014). Acute thermal stressor increases glucocorticoid response but minimizes testosterone and locomotor performance in the cane toad (*Rhinella marina*). *PloS One, 9*(3), e92090. doi:10.1371/journal.pone.0092090

Neaves, L. E., Zenger, K. R., Robert, I. T. P., Mark, D. B. E. & Riddle, B. (2012). Impact of Pleistocene aridity oscillations on the population history of a widespread, vagile Australian mammal, *Macropus fuliginosus*. *Journal of Biogeography, 39*(8), 1545-1563. doi:10.1111/j.1365-2699.2012.02702.x

Newsome, A., Merchant, J., Bolton, B. & Dudzinski, M. (1977). Sexual dimorphism in molar progression and eruption in the agile wallaby. *Wildlife Research, 4*(1), 1-5. doi:10.1071/WR9770001

Nguyen, N., Saggese, M. D. & Eng, C. (2018). Analysis of historical medical records of California condors (*Gymnogyps californianus*) admitted for lead exposure to the Los Angeles Zoo and Botanical Gardens between 1997 and 2012: A case series study. *Journal of Zoo and Wildlife Medicine, 49*(4), 902-911. doi:10.1638/2018-0022.1

Noback, C. V. (1930). Report of the veterinarian. *Animal Kingdom Annual Report. 34th* (pp. 42-44). New York, NY: New York Zoological Society.

Nocard, E. & Le Clainche, E. (1898). Malades microbiennes des animaux. *Cited by Mouquet (1923)*.

Noordzij, M., Dekker, F. W., Zoccali, C. & Jager, K. J. (2010). Measures of disease frequency: Prevalence and incidence. *Nephron Clinical Practice, 115*, 17-20. doi:10.1159/000286345

Nowak, R. M. (2018). *Walker's mammals of the world: monotremes, marsupials, afrotherians, xenarthrans, and sundatherians*. Baltimore, MA: Johns Hopkins University Press.

Nowak, R. M. & Walker, E. P. (1999). *Walker's mammals of the world* (6th ed. Vol. 1). Baltimore, MD: John Hopkins University Press.

O'Regan, H. J. & Kitchener, A. C. (2005). The effects of captivity on the morphology of captive, domesticated and feral mammals. *Mammal Review, 35*(3-4), 215-230. doi:10.1111/j.1365-2907.2005.00070.x

Oliphant, J. C., Parsons, R. & Smith, G. R. (1984). Aetiological agents of necrobacillosis in captive wallabies. *Research in Veterinary Science, 36*(3), 382-384.

Olivkov, B. M. & Nossoova, O. A. (1940). *Cases of necrobacillosis in Artiodactyla and Macropus in the Moscow Zoological Gardens.* Paper presented at the Proceedings of the Moscow Zoological Park (pp. 322-335), Moscow, Russia: Moscow Zoological Park.

Oostman, O. & Smego, R. A. (2005). Cervicofacial actinomycosis: Diagnosis and management. *Current Infectious Disease Reports, 7*(3), 170-174. doi:10.1007/s11908-005-0030-0

Ord, T. J., Evans, C. S. & Cooper, D. W. (1999). Nocturnal behaviour of the parma wallaby, *Macropus parma* (Marsupialia : Macropodoidea). *Australian Journal of Zoology, 47*(2), 155-167. doi:10.1071/ZO98047

Oz, H. S. & Puleo, D. A. (2011). Animal models for periodontal disease. *Journal of Biomedicine & Biotechnology, 2011*, 754857. doi:10.1155/2011/754857

Padalino, B., Hall, E., Raidal, S., Celi, P., Knight, P., Jeffcott, L. & Muscatello, G. (2015). Health problems and risk factors associated with long haul transport of horses in Australia. *Animals, 5*(4), 1296-1310. doi:10.3390/ani5040412

Penzhorn, B. L. (1984). Dental abnormalities in free-ranging Cape mountain zebras (*Equus zebra zebra*). *Journal of Wildlife Diseases, 20*(2), 161-166. doi:10.7589/0090-3558-20.2.161

Phillips, C., Higgins, D., Herbert, C. & Spielman, D. (2012). *Investigating the efficacy of '5 in 1' vaccines in macropods.* Paper presented at the Australian Wildlife Rehabilitation Conference, Townsville, Queensland.

Pihlstrom, B. L., Michalowicz, B. S. & Johnson, N. W. (2005). Periodontal diseases. *The Lancet, 366*(9499), 1809–1820 doi:10.1016/S0140-6736(05)67728-8

Plowman, A., Hosey, G., Stevenson, M. & Whitehouse-Tedd, K. (2013). Surveys and questionnaires. In A. Moss (Ed.), *Handbook of zoo research: Guidelines for conducting research in zoos* (pp. 134-150). London, United Kingdom: BIAZA.

Plumb, D. C. (2018). Azithromycin *Veterinary drug handbook* (9th, pp. 158-162 ed.). Stockholm, WI: Pharma Vet Publishing.

Poelma, F. (1964). Diseases in marsupials in captivity. *Tijdschrift voor Diergeneeskunde, 89*(Suppl. No. 1), 149-155.

Poole, W., Carpenter, S. & Wood, J. (1982). Growth of grey kangaroos and the reliability of age determination from body measurements II. The western grey kangaroos, *Macropus fuliginosus fuliginosus*, *M. f. Melanops* and *M. f. Ocydromus*. *Wildlife Research, 9*(2), 203-212. doi:10.1071/WR9820203

Princée, F. P. G. (1998). *Genetic management ofsmall animal populations in zoos and wildlife reserves.The use of genome models in estimation of geneticvariation and the effects of social structures.* Unpublished PhD thesis, University of Groningen, Groningen, The Netherlands.

Princée, F. P. G. (2016). *Exploring studbooks for wildlife management and conservation* (Vol. 17). Cham, Switzerland: Springer International Publishing.

Probst, C. & Matschei, C. (2008). Mixed-species exhibits with mammals in central European zoos. *International Zoo News, 55*(6), 324-347.

R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Rees, P. A. (2004). Low environmental temperature causes an increase in stereotypic behaviour in captive Asian elephants (*Elephas maximus*). *Journal of Thermal Biology, 29*(1), 37-43. doi:10.1016/j.jtherbio.2003.10.004

Rees, P. A. (2011). *An introduction to zoo biology and management*. Chichester, United Kingdom: Wiley-Blackwell.

Reiss, A. & Woods, R. (2011). *National zoo biosecurity manual*. Canberra, ACT: Australian Government Department of Agriculture, Fisheries and Forestry.

Rendle, J., McCormick, W. & Ward, S. (2018). Behaviour and enclosure use of captive parma wallabies (Macropus parma): an assessment of compatibility within a mixed-species exhibit. *Journal of Zoo and Aquarium Research, 6*(2), 137-142.

Rindfuss, R. R., Choe, M. K., Tsuya, N. O., Bumpass, L. L. & Tamaki, E. (2015). Do low survey response rates bias results? Evidence from Japan. *Demographic Research, 32*, 797-828. doi:10.4054/DemRes.2015.32.26

Roberts, D. S. & Egerton, J. R. (1969). The aetiology and pathogenesis of ovine foot-rot: II. The pathogenic association of *Fusiformis nodosus* and *F. necrophorus*. *Journal of Comparative Pathology, 79*(2), 217-227. doi:10.1016/0021-9975(69)90008-5

Roeder, B. L., Chengappa, M. M., Lechtenberg, K. F., Nagaraja, T. G. & Varga, G. A. (1989). *Fusobacterium necrophorum* and *Actinomyces pyogenes* associated facial and mandibular abscesses in blue duiker. *Journal of Wildlife Diseases, 25*(3), 370-377. doi:10.7589/0090-3558-25.3.370

Ross, T. D. (2003). Accurate confidence intervals for binomial proportion and Poisson rate estimation. *Computers in Biology and Medicine, 33*(6), 509-531. doi:10.1016/S0010-4825(03)00019-2

Rushen, J. (2000). Some issues in the interpretation of behavioural responses to stress *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 23-42). Wallingford, United Kingdom: CABI Publishing.

Ryser-Degiorgis, M. P. (2013). Wildlife health investigations: needs, challenges and recommendations. *BMC Veterinary Research, 9*, 223-240. doi:10.1186/1746-6148-9-223

Samuel, J. & Fowler, M. (1981). *Bacterial flora of the mouth and of lesions of the jaw in macropods.* Paper presented at the Wildlife Diseases of the Pacific Basin and Other Countries. Proceedings of the 4th International Conference of the Wildlife Disease Association (pp. 56-58), Sydney, New South Wales.

Samuel, J. L. (1982). The normal flora of the mouths of macropods (Marsupialia: Macropodidae). *Archives of Oral Biology, 27*(2), 141-146. doi:10.1016/0003-9969(82)90134-0

Samuel, J. L. (1983). Jaw disease in macropod marsupials: Bacterial flora isolated from lesions and from the mouths of affected animals. *Veterinary Microbiology, 8*(4), 373-387. doi:10.1016/0378-1135(83)90050-0

Sanson, G. (1980). The morphology and occlusion of the molariform cheek teeth in some Macropodinae (Marsupialia: Macropodidae). *Australian Journal of Zoology, 28*(3), 341-365. doi:10.1071/ZO9800341

Sanson, G. (1989). Morphological adaptations of teeth to diets and feeding in the Macropodoidea. In G. Grigg, P. Jarman, & I. Hume (Eds.), *Kangaroos, wallabies and rat-kangaroos* (Vol. 1, pp. 151-168). Sydney, New South Wales: Surrey Beathy and Sons.

Sanson, G. D., Kerr, S. A. & Gross, K. A. (2007). Do silica phytoliths really wear mammalian teeth? *Journal of Archaeological Science, 34*(4), 526-531. doi:10.1016/j.jas.2006.06.009

Schaefer, D. R. & Dillman, D. A. (1998). Development of a standard e-mail methodology: Results of an experiment. *Public Opinion Quarterly, 62*(3), 378-397. doi:10.1086/297851

Schneider, H. E., Berger, G., Dathe, F. & Gensch, W. (1976). Erkrankungen Australischer tiere im Zoologischen Garten Dresden. *VISZ, 18*, 5-12.

Schröder, V. H. E. & Ippen, R. (1976). *Beitrag zu den Erkrankungen der Kanguruhs.* Paper presented at the Erkrankungen der Zootiere. Verhandlungsbericht des XVIII Internationalen Symposiums uber die Erkrankungen der Zootiere, Innsbruck, Austria.

Schulte-Hostedde, A. I. & Mastromonaco, G. F. (2015). Integrating evolution in the management of captive zoo populations. *Evolutionary Applications, 8*(5), 413-422. doi:10.1111/eva.12258

Schultz, D. J., Whitehead, P. J. & Taggart, D. A. (2006). Review of surrogacy program for endangered victorian brush-tailed rock wallaby (*Petrogale penicillata*) with special reference to animal husbandry and veterinary considerations. *Journal of Zoo and Wildlife Medicine, 37*(1), 33-39. doi:10.1605/01.301-0001210255.2007

Scott, H. H. & Camb, H. (1925). A streptothrix disease of wallabies. *Proceedings of the Zoological Society of London, 95*(3), 799-814. doi:10.1111/j.1469-7998.1925.tb07106.x

Seimens Medical. (2007). *SOMATOM Emotion 6/16-slice configuration: Application Guide - Protocols, Principles, Helpful Hints. Software Version syngo CT 2007E*. Erlangen, Germany: Seimens.

Sergeant, E. (2018). *Epitools epidemiological calculators*. Retrieved from http://epitools.ausvet.com.au

Shah, A., Memon, M., Ali, G., Khan, A., Yuan, X., Liu, Q. & Shao, T. (2016). Careful surgery, management and treatment of bovine calf actinomycosis (lumpy jaw). *Academia Journal of Agricultural Research, 4*(2), 86-90. doi:10.15413/ajar.2015.0186

Sharman, G. B., Frith, H. J. & Calaby, J. H. (1964). Growth of the pouch young, tooth eruption and age determination in the red kangaroo, *Megaleia rufa*. *Wildlife Research, 9*(1), 20-49. doi:10.1071/CWR9640020

Sherwen, S. L., Hemsworth, P. H., Butler, K. L., Fanson, K. V. & Magrath, M. J. L. (2015). Impacts of visitor number on kangaroos housed in free-range exhibits. *Zoo Biology, 34*(4), 287-295. doi:10.1002/zoo.21226

Silk, M. J., Croft, D. P., Delahay, R. J., Hodgson, D. J., Boots, M., Weber, N. & McDonald, R. A. (2017). Using social network measures in wildlife disease ecology, epidemiology, and management. *BioScience, 67*(3), 245-257. doi:10.1093/biosci/biw175

Sillmann, J., Croci-Maspoli, M., Kallache, M. & Katz, R. W. (2011). Extreme cold winter temperatures in europe under the influence of North Atlantic atmospheric blocking. *Journal of Climate, 24*(22), 5899-5913. doi:10.1175/2011jcli4075.1

Slots, J. & Ting, M. (2002). Systemic antibiotics in the treatment of periodontal disease. *Periodontology 2000, 28*(1), 106-176. doi:10.1034/j.1600-0757.2002.280106.x

Smith, G., Oliphant, J. C. & Parsons, R. (1984). The pathogenic properties of *Fusobacterium* and *Bacteroides* species from wallabies and other sources. *Journal of Hygiene, 92*(02), 165-175. doi:10.1017/S0022172400064184

Smith, G. R. & Thornton, E. A. (1993). Pathogenicity of Fusobacterium necrophorum strains from man and animals. *Epidemiology and Infection, 110*(3), 499-506. doi:10.1017/S0950268800050925

Smith, G. R., Turner, A. & Cinderey, R. (1986). Susceptibility of wallabies to *Fusobacterium necrophorum*. *Veterinary Record, 118*(25), 691-693. doi:10.1136/vr.118.25.691

Smith, G. R., Turner, A., Murray, L. G. & Oliphant, J. C. (1985). The weak immunogenicity of *Fusobacterium necrophorum*. *Journal of Hygiene, 95*(1), 59-68. doi:10.1017/S0022172400062288

Smith, G. R., Wallace, L.M. and Noakes, D.E. (1990). Experimental observations on the pathogenesis of necrobacillosis. *Epidemiology and Infection, 104*, 73-78. doi:10.1017/S0950268800054546

Smith, J. A. (2009). Macropod nutrition. *Veterinary Clinics of North America: Exotic Animal Practice, 12*(2), 197-208. doi:10.1016/j.cvex.2009.01.010

Sós, E., Szigeti, A., Fok, É., Molnár, V., Erdélyi, K., Perge, E., Biksi, I. & Gál, J. (2012). Toxoplasmosis in tammar wallabies (*Macropus eugenii*) in the Budapest Zoo and Botanical Garden (2006–2010). *Acta Veterinaria Hungarica, 60*(3), 361-370. doi:10.1556/AVet.2012.031

Soto, E., Arauz, M., Gallagher, C. A. & Illanes, O. (2014). *Nocardia cyriacigeorgica* as the causative agent of mandibular osteomyelitis (lumpy jaw) in a cat. *Journal of Veterinary Diagnostic Investigation, 26*(4), 580-584. doi:10.1177/1040638714533117

Sotohira, Y., Okui, H., Suzuki, K., Asakawa, M. & Sano, T. (2017a). Association between the levels of stress markers and the onset of kangaroo disease (lumpy jaw disease) in captive kangaroos. *Journal of Zoo Biology, 1*(1), 17-20.

Sotohira, Y., Suzuki, K., Sano, T., Arai, C., Asakawa, M. & Hayashi, H. (2017b). Stress assessment using hair cortisol of kangaroos affected by the lumpy jaw disease. *Journal of Veterinary Medical Science, 79*(5), 852-854. doi:10.1292/jvms.16-0633

Sotohira, Y., Suzuki, K., Sasaki, H., Sano, T., Tsuchiya, M., Suzuki, Y., Shimamori, T., Tsukano, K., Sato, A. & Yokota, H. (2017c). Plasma endotoxin activity in kangaroos with oral necrobacillosis (lumpy jaw disease) using an automated handheld testing system. *Journal of Veterinary Medical Science, 78*(6), 971-976. doi:10.1292/jvms.15-0513

Species360. (2018). *Species360, ZIMS (Version 2.3 Updated: 2016, January 25th). Zoological Information Management System*. Sponsoring Institution Perth Zoo, Retrieved from http://zims.Species360.org on January 1st 2019.

Speksnijder, D. C., Graveland, H., Eijck, I. A., Schepers, R. W., Heederik, D. J., Verheij, T. J. & Wagenaar, J. A. (2017). Effect of structural animal health planning on antimicrobial use and animal health variables in conventional dairy farming in the Netherlands. *Journal of Dairy Science, 100*(6), 4903-4913. doi:10.3168/jds.2016-11924

Staker, L. (2014). *Macropod Husbandry, Healthcare and Medicinals.* (1st ed. Vol. 1 & 2). n.p.: Lynda Staker.

Stallknecht, D. (2007). Impediments to wildlife disease surveillance, research, and diagnostics. In J. E. Childs, J. S. Mackenzie, & J. A. Richt (Eds.), *Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and Consequences of Cross-Species Transmission* (pp. 445-461). Berlin, Germany: Springer.

Steidl, R. J., Hayes, J. P. & Schauber, E. (1997). Statistical power analysis in wildlife research. *Journal of Wildlife Management, 61*(2), 270-279.

Steinberg, D. & Friedman, M. (1999). Dental drug-delivery devices: local and sustained release applications. *Critical Reviews™ in Therapeutic Drug Carrier Systems, 16*(5), 425–459. doi:10.1615/CritRevTherDrugCarrierSyst.v16.i5.10

Steinberg, D. & Rothman, M. (1996). Antibacterial effect of chlorhexidine on bacteria adsorbed onto experimental dental plaque. *Diagnostic Microbiology and Infectious Disease, 26*, 109-115. doi:10.1016/S0732-8893(96)00201-5

Strahan, R. (1995). *The mammals of Australia* (2nd ed.). Sydney, New South Wales: Reed Books.

Supranoto, S. C., Slot, D. E., Addy, M. & Van der Weijden, G. A. (2015). The effect of chlorhexidine dentifrice or gel versus chlorhexidine mouthwash on plaque, gingivitis, bleeding and tooth discoloration: A systematic review. *International Journal of Dental Hygiene, 13*, 83-92. doi:10.1111/idh.12078

Takahashi, N. (2005). Microbial ecosystem in the oral cavity: Metabolic diversity in an ecological niche and its relationship with oral diseases. *International Congress Series, 1284*, 103-112. doi:10.1016/j.ics.2005.06.071

Takai, S., Ohbushi, S., Koike, K., Tsubaki, S., Oishi, H. & Kamada, M. (1991). Prevalence of virulent Rhodococcus equi in isolates from soil and feces of horses from horse-breeding farms with and without endemic infections. *Journal of Clinical Microbiology, 29*(12), 2887-2889.

Tancawan, A. L., Pato, M. N., Abidin, K. Z., Asari, A., Thong, T. X., Kochhar, P., Muganurmath, C., Twynholm, M. & Barker, K. (2015). Amoxicillin/clavulanic acid for the treatment of odontogenic infections: a randomised study comparing efficacy and tolerability versus clindamycin. *International Journal of Dentistry, 2015*, 472470-472479. doi:10.1155/2015/472470

Taylor, M. R. H., Wilson, P., Smyth, L., English, L. & Keane, C. T. (1978). Laboratory investigations in the "Lumpy Jaw" of macropods. *Veterinary Science Communications, 2*(1), 131-136. doi:10.1007/BF02291441

Terlouw, E. M., Schouten, W. & Ladewig, J. (1997). Physiology. In M. C. Appleby & B. O. Hughes (Eds.), *Animal Welfare* (pp. 143-158). Wallingford, United Kingdom: CAB International.

Thomas, C., Finn, M., Twigg, L., Deplazes, P. & Thompson, R. C. A. (1997). Microsporidia (*Encephalitozoon cuniculi*) in wild rabbits in Australia. *Australian Veterinary Journal, 75*(11), 808-810. doi:10.1111/j.1751-0813.1997.tb15658.x

Thomas, O. (1888). *Catalogue of the Marsupialia and Monotremata in the collection of the British Museum (Natural History)*. In B. M. London (Ed.). London, United Kingdom: Order of the Trustees.

Thrusfield, M. V. & Christley, R. (2018). *Veterinary epidemiology* (4th ed.). Chichester, United Kingdom: Wiley Blackwell.

Tomlinson, A. R. & Gooding, C. G. (1954). A kangaroo disease. Investigations into 'lumpy jaw' on the Murchison, 1954. *Journal of the Department of Agriculture, Western Australia, 3*, 715-718.

Tsvetaeva, N. P. (1941). Diseases of animals in the Moscow Zoological Park. *Trudy Moscov Zooparka, 2*, 153.

Tucker, R. & Millar, R. (1953). Outbreak of nocardiosis in masupials in the Brisbane Botanical Gardens. *Journal of Comparative Pathology, 63*(2), 143-146. doi:10.1016/S0368-1742(53)80016-0

Tyndale-Biscoe, C. H. (2005). *Life of marsupials*. Melbourne, Victoria: CSIRO Publishing.

Valour, F., Sénéchal, A., Dupieux, C., Karsenty, J., Lustig, S., Breton, P., Gleizal, A., Boussel, L., Laurent, F. & Braun, E. (2014). Actinomycosis: Etiology, clinical features, diagnosis, treatment, and management. *Infection and Drug Resistance, 7*, 183-197. doi:10.2147/IDR.S39601

Van Dyck, S. & Strahan, R. (2008). *The mammals of Australia* (3rd ed.). Sydney, New South Wales: New Holland Publishers.

Van Gelder, M. M., Bretveld, R. W. & Roeleveld, N. (2010). Web-based questionnaires: the future in epidemiology? *American Journal of Epidemiology, 172*(11), 1292-1298. doi:10.1093/aje/kwq291

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: Causes and threats. *Pharmacy and Therapeutics, 40*(4), 277-283.

Vogelnest, L. (2003). *Captive management of Australian marsupials.* Paper presented at the Proceedings of the North American Veterinary Conference, Orlando, FL.

Vogelnest, L. (2015). Marsupialia (Marsupials) In R. E. Miller & M. E. Fowler (Eds.), *Fowler's Zoo and Wild Animal Medicine, Volume 8* (Vol. 8, pp. 255-274). St. Louis, MO: W.B. Saunders.

Vogelnest, L. & Portas, T. (2008). Macropods. In L. Vogelnest & R. Woods (Eds.), *Medicine of Australian Mammals*. Collingwood, Victoria: CSIRO Publishing.

Walker, B. & McKinnon, R. (2002). *Lumpy jaw and wooden tongue in cattle*. Sydney, New South Wales: Department of Primary Industries Retrieved from https://www.dpi.nsw.gov.au/animals-and-livestock/beef-cattle/health-and-disease/bacterial-diseases/lumpy-jaw-wooden-tongue.

Wallach, J. D. (1971). Lumpy jaw in captive kangaroos. *International Zoo Yearbook, 11*(1), 13. doi:10.1111/j.1748-1090.1971.tb01827.x

Wallach, J. D. & Boever, W. J. (1983). *Diseases of exotic animals. Medical and surgical management*. Philadelphia, PA: W.B. Saunders.

Warburton, N. M. (2009). Comparative jaw muscle anatomy in kangaroos, wallabies, and rat-kangaroos (Marsupialia: Macropodoidea). *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology, 292*(6), 875-884. doi:10.1002/ar.20905

Ward, S. J. & Melfi, V. (2013). The implications of husbandry training on zoo animal response rates. *Applied Animal Behaviour Science, 147*(1-2), 179-185. doi:10.1016/j.applanim.2013.05.008

Watson, M. K., Papich, M. G. & Chinnadurai, S. K. (2017). Pharmacokinetics of intravenous clindamycin phosphate in captive Bennett's wallabies (*Macropus rufogriseus*). *Journal of Veterinary Pharmacology and Therapeutics, 40*(6), 682-686. doi:10.1111/jvp.12421

Watts, P. S. & McLean, S. J. (1956). *Bacteroides* infection in kangaroos. *Journal of Comparative Pathology and Therapeutics, 66*, 159-162. doi:10.1016/S0368-1742(56)80017-9

Weigl, R. (2005). *Longevity of mammals in captivity; from the living collections of the world*. Stuttgart, Germany: Kleine Senckenberg-Reihe.

Wenker, C. J., Stich, H., Müller, M. & Lussi, A. (1999). A retrospective study of dental conditions of captive brown bears (*Ursus arctos* spp.) compared with free-ranging Alaskan grizzlies (*Ursus arctos horribilis*). *Journal of Zoo and Wildlife Medicine, 30*(2), 208-221.

Whittier, W. D. & Umberger, S. H. (2009). Control, treatment and elimation of foot rot from sheep. *Virginia Cooperative Extension*, 1-4.

Whittington, R. J. (1995). Observations on the indirect transmission of virulent ovine footrot in sheep yards and its spread in sheep on unimproved pasture. *Australian Veterinary Journal, 72*(4), 132.

Wiggs, R. & Lobprise, H. (1994). Acute and chronic alveolitis/osteomyelitis ("lumpy jaw") in small exotic ruminants. *Journal of Veterinary Dentistry, 11*(3), 106-109.

Wildlife Health Australia. (2018). *National Wildlife Biosecurity Guidelines*. Sydney, New South Wales: Wildlife Health Australia.

Wilson, P., Taylor, M., Keane, C., English, L. & Allen, M. (1980). Metronidazole in the treatment of lumpy-jaw in a Bennett's wallaby. *Veterinary Record, 107*(5), 111-112.

Winter, J. R., Kaler, J., Ferguson, E., KilBride, A. L. & Green, L. E. (2015). Changes in prevalence of, and risk factors for, lameness in random samples of English sheep flocks: 2004 – 2013. *Preventive Veterinary Medicine, 122*(1-2), 121-128. doi:10.1016/j.prevetmed.2015.09.014

Witcomb, L. A., Green, L. E., Kaler, J., Ul-Hassan, A., Calvo-Bado, L. A., Medley, G. F., Grogono-Thomas, R. & Wellington, E. M. H. (2014). A longitudinal study of the role of *Dichelobacter nodosus* and *Fusobacterium necrophorum* load in initiation and severity of footrot in sheep. *Preventive Veterinary Medicine, 115*(1–2), 48-55. doi:10.1016/j.prevetmed.2014.03.004

Witt, J. & Green, L. (2018). Development and assessment of management practices in a flock-specific lameness control plan: A stepped-wedge trial on 44 English sheep flocks. *Preventive Veterinary Medicine, 157*, 125-133. doi:10.1016/j.prevetmed.2018.06.013

Wobester, G. A. (2006). *Essentials of disease in wild animals*. Ames, IA: Blackwell Publishing.

Woinarski, J. C., Burbidge, A. A. & Harrison, P. L. (2015). Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement. *Proceedings of the National Academy of Sciences, 112*(15), 4531-4540. doi:10.1073/pnas.1417301112

Woinarski, J. C. Z., Burbidge, A. A. & Harrison, P. (2014). *The action plan for Australian mammals 2012*. Melbourne, Victoria: CSIRO Publishing.

Wong, J., Wang, K., Henry, P., Maloney, S. & Noble, P. (2018). The physiological properties of the kangaroo airway. *Respirology, 23*(S1), 76.

Wright, S. (1921). Systems of matings (Part I - V). *Genetics, 6*, 111-178.

Young, E. (1965). Pasteurellosis in the kangaroo. *International Zoo Yearbook, 5*(1), 185-185. doi:10.1111/j.1748-1090.1965.tb01635.x

Zhou, H., Bennett, G. & Hickford, J. G. H. (2009). Variation in *Fusobacterium necrophorum* strains present on the hooves of footrot infected sheep, goats and cattle. *Veterinary Microbiology, 135*(3–4), 363-367. doi:10.1016/j.vetmic.2008.09.084

Ziembicki, M. & Porolak, G. (2016). Huon tree kangaroo. *Dendrolagus matschiei*. The IUCN Red List of Threatened Species 2016, e.T6433A21956650. Retrieved from https://www.iucnredlist.org/species/6433/21956650 doi:10.2305/IUCN.UK.2016-2.RLTS.T6433A21956650.en

APPENDICES

APPENDIX A: Letter inviting zoos to participate in the retrospective study of lumpy jaw

Murdoch
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Jessica Rendle
Conservation Medicine Program
College of Veterinary Medicine
School of Veterinary and Life Sciences
Murdoch University
Murdoch, 6150
Western Australia
T. +61 (0)8 9360 6718
M. +61 (0)449 838710
E. j.rendle@murdoch.edu.au

8th April 2016

Dear Dr.............................,

Re: Request for participation in macropod lumpy jaw PhD study

As part of a larger PhD research project investigating lumpy jaw in captive macropods, we are requesting the support of zoological institutions, from across Australia and Europe, to assist with a detailed investigation of the effects of management procedures on macropod health and behaviour. Despite the high incidence of lumpy jaw reported anecdotally in zoo macropods worldwide, few epidemiological studies have investigated the risk factors associated with the expression of the disease. It is hoped that this research will provide useful information to aid in the optimal management of captive macropod populations, through an improved understanding of housing and husbandry risk factors, effective treatment regimens and preventative measures associated with lumpy jaw in macropods.

Associated with this study, I hope that it will be possible for me to visit XXXX Zoo during the spring/summer of 2016, for approximately 10 days and have access to historic (~1995-2015) and current veterinary records, and those associated with macropod housing and husbandry. In addition, I will be keen to take measurements from current macropod enclosures (including photography). All techniques incorporated in this study are based on having as little impact on zoo personnel and their animals as possible. To assist with the extraction of records-based data, specific training, undertaken with our collaborating zoo (XXXX Zoo) will provide a good understanding of the ZIMS and Medical ZIMS prior to attending XXXX Zoo.

Results from this investigation will provide baseline data required for the development of recommendations for the management of macropods in captivity, providing keepers with a practical framework for the prevention of this disease. In addition, veterinarians will be equipped with data relating to the most effective treatment regime for lumpy jaw in a captive environment. There are significant welfare benefits to this study, including the acquisition of a deeper understanding of housing and husbandry requirements and a potential decrease in morbidity and mortality rates associated with this disease; factors that will be of benefit to the welfare of captive macropods across Australia and Europe.

The study has been provided with OUTRIGHT approval by the Murdoch University Animal Ethics Committee (Permit Number: R2754/15) and Human Research Ethics Committee (Project No. 2015/182). We have also received full Research Committee and Animal Ethics Committee approval at XXXX Zoo. In addition, we have gained the support of the leading zoological bodies including British and Irish Association of Zoos and Aquariums (see attached letter), European Association of Zoos and Aquaria, the European Marsupial and Monotreme Taxon Advisory Group, the Zoo and Aquarium Association, the Australian Mammals Veterinary Advisory Group and Veterinary

CRICOS Provider Code: 00125J
ABN 61 616 369 313

APPENDIX A: Letter inviting zoos to participate in the retrospective study of lumpy jaw (continued)

Murdoch
UNIVERSITY

Specialist Advisory Group. Supervision is provided by a team of world-renowned experts in Conservation Medicine based at Murdoch University, Western Australia. The team is led by Associate Professor Kristin Warren with co-supervision from wildlife veterinarians and epidemiologist, Dr Bethany Jackson; a veterinarian specialising in veterinary dentistry, Dr Lian Yeap; and Zoo Biologist, Dr Samantha Ward of Nottingham Trent University in the UK.

We appreciate the time and effort that is involved in approving research projects undertaken in zoological establishments and would be happy to work with you to ensure institutional approval is permitted for the study to take place at your establishment. In return for your participation and support, we would offer co-authorship (individual zoo personnel or institution) on publications that arise from the findings of this study.

The support of XXXX Zoo would be an asset to our investigation and we appreciate your time in considering our request.

Yours sincerely,

Jessica Rendle BSc (Hons) MSc ATLS

CRICOS Provider Code: 00125J
ABN 61 616 369 313

APPENDIX B: Survey distributed to zoos across Australia and Europe

Information and consent

Thank you for taking the time to complete this questionnaire, the information you provide is incredibly valuable and very much appreciated. Your feedback is welcome at any stage.

The purpose of this questionnaire is to gather information on the current epidemiological status of lumpy jaw in captive macropods, evaluate the efficacy of treatments used for the condition, and determine housing and husbandry practices that may be associated with the onset of this disease. This questionnaire forms part of a larger PhD project that is researching the influence that housing and husbandry has on lumpy jaw in macropods housed in zoological institutions across Australia and Europe.

Lumpy jaw is a painful condition that affects several macropod species and it is a well-recognised cause of morbidity and mortality in captive macropods worldwide. Our research aims to identify housing and husbandry procedures and practices that may have an associative role with cases of lumpy jaw in the zoo environment, in addition to an investigation of disease epidemiology and assessment of the success of treatments used in zoos. Collectively, this information will be used to create revised husbandry guidelines aimed at reducing cases of lumpy jaw in captive populations of macropods.

Please indicate that you have read and understood the 'Participant Information Statement' (provided as a separate attachment with the email) and that you consent to completing this questionnaire.

☐ **Yes**
☐ **No**

We would like to remind you that having access to your macropod veterinary and husbandry records whilst completing this questionnaire will greatly assist you.

This study has been approved by the Murdoch University Human Research Ethics Committee (Approval 2015/182). If you have any reservation or complaint about the ethical conduct of this research, and wish to talk with an independent person, you may contact Murdoch University's Research Ethics & Integrity on Tel. 08 9360 6677 (+61 8 9360 6677 for overseas studies) or e-mail ethics@murdoch.edu.au. Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

Lumpy jaw in zoo macropods Page 1

APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

Questionnaire for Research Project: Lumpy Jaw in Zoo Macropods

All the following questions apply to the animals kept at your institution only and where possible data should be verified by record searches.

Section 1 – Macropod Housing and Husbandry

1. Where is your zoological institution located? Please specify the country and the closest town/city (e.g. Sydney, Australia).

   ....

2. What is your role at the zoo? Please tick all those that apply.

   ☐ Veterinarian based onsite at the zoo

   ☐ Veterinarian based offsite

   ☐ Veterinary Nurse based onsite at the zoo

   ☐ Veterinary Nurse based offsite

   ☐ Other – please specify below

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3. Which types of macropods do you hold and how do you house them? Please complete the table below for each enclosure.

Enclosure name/ID	Macropod species You may select more than one.	Sex ratio per species (M:F:Unknown)	Ratio of adults to juveniles per species (adult:juvenile)	Non-macropod species and approximate numbers
Example BC023	Macropus rufus Macropus giganteus	1:2:1 1:3:0	3:1 4:0	Emu (6)

Please indicate the source of information for completing this table: Zoo Records: ☐ Zoo Staff: ☐

Comments:

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Lumpy jaw in zoo macropods | Page 2

APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

4. For each macropod group described above, please give the details of the enclosure environment. (Visitor access = visitors able to walk through the enclosure). The *Enclosure Name/ID* section is auto-filled from the answers provided in question 3.

Enclosure name/ID (Auto-filled from question 3)	Approximate enclosure size (ideally length x width) or approx. m^2 or ha	Primary ground surface (Grass, sand, leaf litter, concrete, soil etc.)	Shelters	Number of feeding stations	Visitor access	
Example BC023	5x5m	Sand and leaf litter	2 small metal huts; bushes	3	☐ Yes	☐ No
					☐ Yes	☐ No
					☐ Yes	☐ No
					☐ Yes	☐ No
					☐ Yes	☐ No
					☐ Yes	☐ No
					☐ Yes	☐ No
					☐ Yes	☐ No
					☐ Yes	☐ No
					☐ Yes	☐ No
					☐ Yes	☐ No

Please indicate the source of information for completing this table: Zoo Records: ☐ Zoo Staff: ☐

Comments:

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5. Please describe the normal cleaning routine for macropod equipment:

	Never	Daily	2-3 times per week	Weekly	Fortnightly	Monthly	Other
Enclosure ground surface	☐	☐	☐	☐	☐	☐	☐
Shelters/internal structures	☐	☐	☐	☐	☐	☐	☐
Feeding bowls/troughs	☐	☐	☐	☐	☐	☐	☐
Water bowls/troughs	☐	☐	☐	☐	☐	☐	☐

Please indicate source of information for completing this table: Zoo Records: ☐ Zoo Staff: ☐

Comments:

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Lumpy jaw in zoo macropods Page 3

APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

6. What cleaning agents/methods are used when cleaning specific areas of the animal enclosure?

Please indicate source of information for completing this table: Zoo Records: ☐ Zoo Staff: ☐

	None	Sweeping	Hosing	Washing with soap or detergent	Use of disinfectant	Other cleaning agents/methods used – please specify in the comments section below
Enclosure ground surface	☐	☐	☐	☐	☐	☐
Shelters/internal structures	☐	☐	☐	☐	☐	☐
Feeding bowls/troughs	☐	☐	☐	☐	☐	☐
Water bowls/troughs	☐	☐	☐	☐	☐	☐

<u>Comments:</u>

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7. How often are tools and equipment cleaned?

	Never	Sometimes/infrequently	Always
Between enclosures	☐	☐	☐
After each use	☐	☐	☐

Please indicate source of information for completing this table: Zoo Records: ☐ Zoo Staff: ☐

<u>Comments:</u>

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8. When moving between enclosures, do members of zoo staff wash/disinfect:

	Never	Sometimes	Always
Their hands	☐	☐	☐
Their footwear	☐	☐	☐

<u>Comments:</u>

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APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

9. Please select the food items that are fed to each species of macropod. Alternatively, you may submit a diet sheet for each species of macropod to the email address – j.rendle@murdoch.edu.au

Species (Please select from the drop down box)	Pellets e.g. Commercial kangaroo grazer pellet	Hay/grass e.g. Lucerne hay, fresh grass	Leaves/browse e.g. Acacia, coprosma, acalypha	Fruit e.g. Bananas, pear, apple	Vegetables e.g. Sweet potato, carrot	Bread e.g. White bread	Other – please specify in the comments section below
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No

Please indicate source of information for completing this table: Zoo Records: ☐ Zoo Staff: ☐

Comments:

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10. Please tick which option best describes your method of delivery for the various constituents of the diet.

	Scatter/ ground fed	Trough/bowl on the ground	Trough/bowl raised off the ground	Hand feed	Individual bowl	Other - please specify in the comments section below
Pellets	☐	☐	☐	☐	☐	☐
Hay/grass	☐	☐	☐	☐	☐	☐
Leaves/browse	☐	☐	☐	☐	☐	☐
Fruit	☐	☐	☐	☐	☐	☐
Vegetables	☐	☐	☐	☐	☐	☐
Bread	☐	☐	☐	☐	☐	☐
Other - please specify in the comments section below	☐	☐	☐	☐	☐	☐

Please indicate source of information for completing this table: Zoo Records: ☐ Zoo Staff: ☐

Comments:

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Lumpy jaw in zoo macropods Page 5

APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

11. Do you provide supplementation in the food or water?

☐ **Yes – please complete the table below** ☐ **No – please go to question 12**

Species (Please select from the drop down box)	Please specify supplement(s) provided (e.g. vitamins [A, B, C, D, E] and minerals [calcium, salt licks])

Comments:

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12. a) Do you offer hand feeding to any of your macropods?

☐ **Yes – please complete question 12b** ☐ **No – please go to question 13**

b) If yes, what does the feed consist of?

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APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

Section 2a – General Veterinary Support and Health Checks of Macropods

13. What is the normal availability of veterinary support at the zoo? Please tick all that apply.

	Daily	Two or more visits weekly	One or more visits monthly	Less than one visits per month	As required	Other - please specify in the comments section below
Veterinarian	☐	☐	☐	☐	☐	☐
Veterinary Nurse	☐	☐	☐	☐	☐	☐

Comments:

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14. What routine health checks by a veterinarian occur on macropods? Please tick all that apply.

	Every 3-6 months	Every 1-2 years	Every 3-5 years	Less than every 5 years	Opportunistically	As required
Visual health check	☐	☐	☐	☐	☐	☐
Under manual restraint	☐	☐	☐	☐	☐	☐
Under general anaesthetic	☐	☐	☐	☐	☐	☐

Comments:

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15. Which of the following would occur during a routine health check of a macropod? (i.e. **NOT** investigating lumpy jaw cases)

	Under manual restraint	Under general anaesthetic
Haematology (cell blood count)	☐	☐
Serum biochemistry	☐	☐
Radiography of teeth and jaw	☐	☐
Oral/dental examination	☐	☐
Weight	☐	☐
Body condition assessment	☐	☐
Other – please list in the comments section below	☐	☐

Comments:

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Lumpy jaw in zoo macropods Page 7

APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

Section 2b: Occurrence of Lumpy Jaw

For the purpose of this study, **our** case definition for lumpy jaw is:

Proliferative bony change or soft tissue inflammation of the maxilla/mandible (lumps), and/or radiographic/visual evidence of osteomyelitis/osteolysis; accompanied by dental disease. There may or may not be demonstrable bacterial involvement through microbial culture.

16. Given **our** case definition for lumpy jaw, have you had any cases of lumpy jaw in the **last five years?**

☐ **Yes – please go to question 17** ☐ **No – please go to question 24**

17. Please provide information regarding animals diagnosed with lumpy jaw in the last five years per species.

Species (Please select from the drop down box)	Number affected	Total number held during this period	Age ratio (Adult: juvenile)	Sex ratio (Male:Female:Unknown)
E.g. Macropus rufus	3	12	3:0	1:2:0

Please indicate source of information for completing this table: Zoo Records: ☐ Zoo Staff: ☐

18. How many of the macropods diagnosed with lumpy jaw over the last five years have died as a result of lumpy jaw?

Died.................................. **Euthanased**..................................

19. In your opinion, which statement best describes the way in which lumpy jaw is usually first detected?

☐ **Routine veterinary health check under general anaesthetic**
☐ **Non-routine health check under general anaesthetic**
☐ **Routine visual veterinary health check**
☐ **Non-routine visual veterinary health check**
☐ **Veterinary health check under manual restraint**
☐ **Keeper observation of behavioural or clinical signs**
☐ **Other - please specify in the comments section below**

Comments:

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APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

20. How was lumpy jaw diagnosed at the zoo in the past 5 years?

	Never	Sometimes	Often	Always
Radiography	☐	☐	☐	☐
Microbial culture	☐	☐	☐	☐
Clinical signs	☐	☐	☐	☐
Other - please specify in the comments section below	☐	☐	☐	☐

Comments:

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Please indicate source of information for completing this section: Zoo Records: ☐ **Zoo Staff:** ☐

21. a) Have you used bacterial culture methods in cases of lumpy jaw?

☐ **Yes – please complete the table below** ☐ **No – please go to questions 22**

b) Please select the bacteria you have cultured from cases of lumpy jaw in the last five years. Please select all that apply.

Bacteria	Never	Sometimes	Often	Always
Actinobacillus muris	☐	☐	☐	☐
Bacteroides denticanoris	☐	☐	☐	☐
Bacteroides nodosus	☐	☐	☐	☐
Bacteroides pyogenes	☐	☐	☐	☐
Bacteroides ruminicola	☐	☐	☐	☐
Cloacibacterium normanense	☐	☐	☐	☐
Enterococcus faecalis	☐	☐	☐	☐
Erwinia billiagiae	☐	☐	☐	☐
Escherichia coli	☐	☐	☐	☐
Fusobacterium necrophorum	☐	☐	☐	☐
Globicatella sulfiditaciens	☐	☐	☐	☐
Mannheimia caviae	☐	☐	☐	☐
Neisseria weaveri	☐	☐	☐	☐
Oribacterium sp.	☐	☐	☐	☐
Peptostreptococcus anaerobius	☐	☐	☐	☐
Porphyromonas gulae	☐	☐	☐	☐
Prevotella heparinolytica	☐	☐	☐	☐
Pseudomonas aeruginosa	☐	☐	☐	☐
Streptococcus uberis	☐	☐	☐	☐
Other – please specify in the comments section below	☐	☐	☐	☐

Comments:

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Please indicate source of information for completing this table: Zoo Records: ☐ **Zoo Staff:** ☐

Lumpy jaw in zoo macropods **Page 9**

APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

22. What treatments have been used to treat lumpy jaw in the last five years? Please tick all that apply.

- ☐ Tooth extraction
- ☐ Apicoectomy
- ☐ Laser therapy
- ☐ Debridement of bone or tooth lesion
- ☐ Antibiotic impregnated beads implanted at the site
- ☐ Oral varnishes/gels e.g. chlorhexidine
- ☐ Systemic antibiotic therapy
- ☐ Nutritional supplementation
- ☐ Other - please specify in the comments section below

Comments:
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Please indicate source of information for completing this section: Zoo Records: ☐ Zoo Staff: ☐

23. What antibiotics have you used in the treatment of lumpy jaw? Please tick all that apply.

- ☐ Aminoglycosides (e.g. neomycin)
- ☐ Chloramphenicol (e.g. chloramphenicol)
- ☐ Fluoroquinolones (e.g. enrofloxacin)
- ☐ Lincosamides (e.g. clindamycin)
- ☐ Nitoimidazoles (e.g. metronidazole)
- ☐ Penicillins (e.g. benzyl penicillin, amoxicillin)
- ☐ Potentiated sulphonamides (e.g. sulphadiazine plus trimethroprim)
- ☐ Sulphonamides (e.g. sulphadiazine)
- ☐ Tetracylcines (e.g. oxytetracycline)
- ☐ Other – please specify in the comments section below

Comments:
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Please indicate source of information for completing this table: Zoo Records: ☐ Zoo Staff: ☐

24. Have you used vaccinations to prevent lumpy jaw?

☐ Yes – please specify what you have used below ☐ No – go to question 25

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25. What do you consider to be important factors in preventing of lumpy jaw?

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Lumpy jaw in zoo macropods Page 10

APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

Thank you for taking the time to complete our questionnaire. If you would like a summary of the results from this questionnaire, please provide your preferred email address in the section below. We would also welcome any comments you may have on the subject; these can be written in the section below or emailed directly to Jess Rendle at j.rendle@murdoch.edu.au.

Comments:

APPENDIX B: Survey distributed to zoos across Australia and Europe (continued)

Macropod species (Subfamily Macropodinae) in the 'drop-down boxes' of the online questionnaire.

1. *Dendrolagus bennettianus* – Bennett's tree kangaroo
2. *Dendrolagus dorianus* – Doria's tree kangaroo
3. *Dendrolagus goodfellowi* – Goodfellow's tree kangaroo
4. *Dendrolagus inustus* – Grizzled tree kangaroo
5. *Dendrolagus lumholtzi* – Lumholtz's tree kangaroo
6. *Dendrolagus matschiei* – Matschie's tree kangaroo
7. *Dendrolagus stottae* – Tenkile tree kangaroo
8. *Dendrolagus stellarum* – Seri's tree kangaroo
9. *Dendrolagus spadix* – Lowland tree kangaroo
10. *Dendrolagus ursinus* – Vogelkop tree kangaroo
11. *Dorcopsis atrata* – Black dorcopsis
12. *Dorcopsis hageni* – White-striped dorcopsis
13. Dorcopsis luctuosa – Grey dorcopsis
14. *Dorcopsis muelleri* – Brown dorcopsis
15. *Dorcopsulus macleayi* – Macleay's dorcopsis
16. *Dorcopsulus vanheurni* – Small dorcopsis
17. *Lagorchestes conspicillatus* – Spectacled hare wallaby
18. *Lagorchestes hirsustus* – Rufous hare wallaby
19. *Macropus agilis* – Agile wallaby
20. *Macropus antilopinus* – Antilopine wallaroo/kangaroo/wallaby
21. *Macropus bernardus* – Black wallaroo
22. *Macropus dorsalis* – Black-striped wallaby
23. *Macropus eugenii* – Tammar wallaby
24. *Macropus fuliginosus* – Western grey kangaroo
25. *Macropus giganteus* – Eastern grey kangaroo
26. *Macropus irma* – Western brush wallaby
27. *Macropus parma* – Parma wallaby
28. *Macropus parryi* – Whiptail wallaby
29. *Macropus robustus* – Common wallaroo
30. *Macropus rufogriseus* – Red-necked (Bennett's) wallaby
31. *Macropus rufus* – Red kangaroo
32. *Onychogalea unguifera* – Northern nail-tail wallaby
33. *Onychogalea fraenata* – Bridled nail-tail wallaby
34. *Petrogale assimilis* – Allied rock wallaby
35. *Petrogale brachyotis* – Short-eared rock wallaby
36. *Petrogale burbidgei* – Monjon
37. *Petrogale coenensis* – Cape York rock wallaby
38. *Petrogale concinna* – Narbelek
39. *Petrogale godmani – Godman's rock wallaby*
40. *Petrogale herberti* – Herbert's rock wallaby
41. *Petrogale inornata* – Unadorned rock wallaby
42. *Petrogale lateralis* – Black-footed rock wallaby
43. *Petrogale mareeba – Mareeba rock wallaby*
44. *Petrogale pencillata* – Brush-tailed rock wallaby
45. *Petrogale persephone* - Proserpine rock wallaby
46. *Petrogale purpureicollis – Purple-necked rock wallaby*
47. *Petrogale rothschildi* – Rothschild's rock wallaby
48. *Petrogale sharmani* – Sharman's rock wallaby
49. *Petrogale xanthopus* – yellow-footed rock wallaby
50. *Setonix brachyurus* - Quokka
51. *Thylogale billardierii* – Tasmanian pademelon
52. *Thylogale browni* – New Guinea pademelon
53. *Thylogale brunii* – Dusky pademelon
54. *Thylogale calabyi* – Calaby's pademelon
55. *Thylogale lanatus* – Mountain pademelon
56. *Thylogale stigmatica* – Red-legged pademelon
57. *Thylogale thetis* – Red-necked pademelon
58. *Wallabia bicolor* – Swamp wallaby

Lumpy jaw in zoo macropods Page 12

APPENDIX C: BIAZA Research Committee letter of support for the lumpy jaw research project

BIAZA
WORKING TOGETHER
FOR WILDLIFE

BIAZA Research Committee

Letter of Support for Research Project

The BIAZA Research Committee promotes good quality basic and applied research by and within BIAZA's member collections.

Following critical consideration of the research proposal and subsequent satisfactory responses by the researcher, the committee has agreed to give a letter of support for this study by Jessica Rendle of Murdoch University.

In the opinion of the BIAZA Research Committee, the outcomes of the project are likely to be relevant and useful to zoos and aquariums

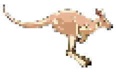
In the interest of scientific training, the BIAZA Research Committee encourages BIAZA members to take part in this research project.

In providing this letter of support, the BIAZA office will have given Jessica Rendle the appropriate contacts from within the BIAZA membership.

Yours faithfully,

Amy Plowman
Chair, BIAZA Research Committee
24th August 2015

APPENDIX D: Participant Information Statement



PARTICIPANT INFORMATION STATEMENT

1) **What is this study about?**
   This questionnaire forms part of the larger PhD project "Epidemiology of the clinical syndrome lumpy jaw in captive macropods: influence of housing and husbandry". Lumpy jaw is a well-recognised cause of morbidity and mortality in captive macropods worldwide suggesting that factors connected with their captive environment, specifically their housing and husbandry, are associated with the development of the disease. Our investigation aims to identify which of these factors may have an associative role with cases of lumpy jaw. To gain a global perspective, our study involves the collection of housing, husbandry and health data of Macropodidae housed in zoos across Australia and Europe. Our aim is to determine the incidence and prevalence of lumpy jaw whilst gaining increased knowledge of the treatments being used to combat this disease.

2) **Who is running the study?**
   The study is being conducted by Jessica Rendle as part of her PhD under the supervision of Associate Professor Kristin Warren, Murdoch University, Conservation Medicine Program, along with co-supervision from wildlife veterinarians and epidemiologists, Drs Bethany Jackson, Carly Holyoake and Lian Yeap. Additional co-supervision is being provided from Zoo Biologist Dr Samantha Ward of Nottingham Trent University in the UK. We are also collaborating with staff at Perth Zoo, Western Australia.

3) **What is involved in the completion of the questionnaire?**
   The questionnaire requires your time, knowledge and experience of lumpy jaw in zoo macropods. We recommend you have access to veterinary and husbandry records to assist you in completing the questionnaire; however there is the option to recall information from personal knowledge or that gained directly from other zoo personnel. You also have the option to submit some information (diet sheets) via email to save time in completing this section in the questionnaire.

4) **What questions will be asked?**
   The questionnaire is divided into two main sections: 1) Macropod Housing and Husbandry, including questions about cleaning routines and diet; 2a) General Veterinary Support and Health Checks including questions about the frequency of health checks carried out at your institution; and 2b) Occurrence of Lumpy Jaw, questions include species diagnosed with the condition and the treatments provided and associated outcomes. There are sections throughout the questionnaire for you to add your own thoughts and comments.

5) **Who can complete the questionnaire?**
   The questionnaire has been developed with the support of zoo professionals and has been written with assumption that it will be completed by the zoo veterinarian. However, the zoo veterinary nurse, registrar, curator or keeper, or someone with knowledge of veterinary and husbandry records, could also complete this questionnaire.

6) **Do I have to be in the study? Can I withdraw from the study once I've started?**
   Participation in this study is voluntary and you may withdraw your consent and stop participating at any time prior to submitting the questionnaire. However, once the questionnaire has been submitted, it may not be possible to withdraw individual institutional data. Please contact the primary researcher Jessica Rendle via email j.rendle@murdoch.edu.au at any time to assist you with this matter.

APPENDIX D: Participant Information Statement (continued)

7) **Are there any risks or costs associated with being in the study?**
There are no risks or costs associated with participation in this study. All data provided will be treated in the strictest confidence and institutions and individuals will not be identified.

8) **What are the benefits of being involved in this study?**
Participation in the study will give you an opportunity to contribute to the improved health and welfare of zoo macropods in Australia, Europe and ultimately worldwide.

9) **What will happen to the information collected during the study?**
The responses you provide in this questionnaire will not be used for any purpose other than this research project, and will remain under the secure care of Murdoch University. The questionnaire is hosted via a link to Murdoch University website, however, the university has strict privacy and security policies and the data submitted in the questionnaire, including any additional documentation, will not be accessible to anyone other than the primary researcher and personnel involved in the study. All responses and respondent's email addresses will remain private. The researcher owns your data. Survey data will be stored securely on servers located at Murdoch University.

Results from this questionnaire may be published, however they will not identify any zoological institution or individual taking part in the survey; your anonymity is assured. Please note that unfortunately, due to the large number of institutions participating in this study, we will not be able to include staff from collaborating institutions as co-authors on publications produced as a result of this research.

10) **Can I tell other people about the study?**
Yes, you are welcome to tell other people about the study. We are also happy for the link to the questionnaire to be distributed to other relevant institutions.

11) **What if I would like further information about the study?**
If at any stage you have questions or concerns regarding the study and/or the questionnaire, please feel free to contact the researchers involved in the study, as detailed below:

Jessica Rendle BSc (Hons) MSc
Conservation Medicine Program
College of Veterinary Medicine
Murdoch University, Western Australia
Tel. +61 (08) 9360 6718
Email: j.rendle@murdoch.edu.auu

Associate Professor Kristin Warren BSc, BVMS (Hons), PhD, Dip ECZM (Wildlife Population Health)
Conservation Medicine Program
College of Veterinary Medicine
Murdoch University, Western Australia
Tel. +61 (08) 9360 2647
Email: k.warren@murdoch.edu.au

Dr Bethany Jackson BVSc MVS (Con Med) PhD
Conservation Medicine Program
College of Veterinary Medicine
Murdoch University, Western Australia
Tel. +61 (08) 9360 6718
Email: b.jackson@murdoch.edu.au

APPENDIX D: Participant Information Statement (continued)

12) Will I be told the results of the study?

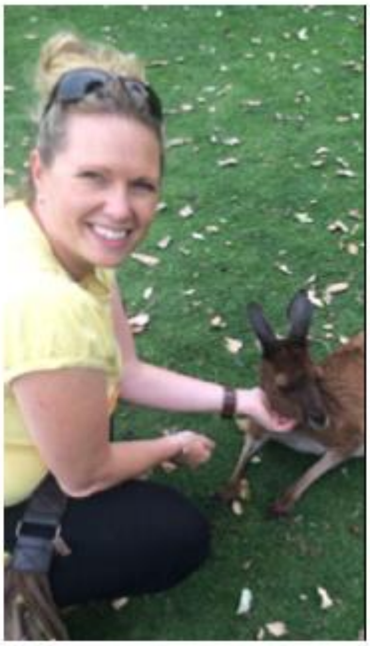
The results are intended for publication in a scientific journal and/or presentation at conferences in Australia and Europe. You will be asked to provide your preferred email address at the end of the questionnaire. You will be notified via email of the results.

13) What if I have a complaint or any concerns about the study?

This study has been approved by the Murdoch University Human Research Ethics Committee (Approval 2015/182). If you have any reservation or complaint about the ethical conduct of this research, and wish to talk with an independent person, you may contact Murdoch University's Research Ethics & Integrity on Tel. 08 9360 6677 (+61 8 9360 6677 for overseas) or e-mail ethics@murdoch.edu.au. Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

Thank you for taking the time to complete this survey, the information you provide is incredibly valuable and very much appreciated. Your feedback is welcome at any stage.

This information sheet is for you to keep



Jessica Rendle

APPENDIX E: Bacterial species listed as 'other' in the survey of lumpy jaw in macropods

Bacterial species	Australia	Europe
Acinetobacter baumannii		X
Acinetobacter sp.		X
Actinobacillus sp.	X	X
Aeromonas hydrophila		X
Aeromonas sobria		X
Aeromonas sp.		X
Bacillus cereus	X	
Bacillus sp.		X
Bacteroides sp.	X	X
Bacteroides stercoris		X
Bacteroides tectus		X
Clostridium clostridiiforme		X
Clostridium perfringens		X
Clostridium tertium		X
Corynebacterium spp.	X	
Entercoccus sp.		X
Enterobacter cloacae	X	
Enterobacter intermedius		X
Enterobacter sakazakii		X
Flavobacterium sp.		X
Fusobacterium nucleatum		X
Fusobacterium sp.		X
Klebsiella pneumonia		X
Klebsiella pneumoniae ssp. *pneumoniae*		X
Klebsiella sp.	X	
Leclercia adecarboxylata		X
Mannheimia hyemolitica	X	
Moraxella sp.	X	X
Mycobacterium avium (not *M. avium paratubuculosis*)		X
Pasteurella sp.		X
Pasteurellaceae[a]	X	
Porphyromonas sp.		X
Propionibacterium spp.		X
Providencia rettgeri		X
Providencias sp.		X
Pseudomonas putida		X
Pseudomonas sp.	X	
Sphingobacterium multivorum		X
Staphylococcus aureus		X
Staphlococcus intermedius		X
Stenotrophomonas maltophila		X
Streptococcus constellatus		X
Streptococcus sp.		X
Wohlfahrtiimonas chitiniclastica		X

[a]**Bacterial 'Family'**

APPENDIX F: Computed Tomography (CT) report describing lumpy jaw lesions in a red kangaroo

The Animal Hospital at Murdoch University

DIAGNOSTIC IMAGING REPORT

Privileged information – not for unauthorized distribution

DESCRIBE LESION in known Lumpy Jaw in Red Kangaroo specimen:

Date of examination: 16/3/17 **Species: Red Kangaroo**

Diagnostic Imaging: CT

Region(s) of Interest / Study Obtained: Skull

Findings: CT study of the skull of red kangaroo with confirmed lesions. Volumetric data sets were acquired of the skull and reformatted into transverse, sagittal and dorsal planes. Additional 3D volume rendered images were also evaluated.

The abnormal mandibular bone was noted bilaterally, with the right mandible more severely affected than the left. The maxilla was unaffected. Three molars and 2 incisors were present bilaterally within the mandible.

Right Mandible: Two areas of the right mandible were noted as abnormal. The first region seen rostrally in the region of the incisor tooth and the second region caudally in the region of the premolar/molar teeth.

Rostrally a focal, expansile, lesion was present within the right mandible at the level of the mandibular symphysis, mental foramen and the right mandibular incisor tooth root. Smooth to slightly irregular new bone was present external to the original cortical margin and the medullary region was filled with increased opacity material throughout the lesion (intraosseous density). A radiolucent space with smooth margins surrounds the tooth root. The cortex within the lesion was reduced in thickness in places, however difficult to define in all areas. The lesion measured approximately 51 mm in length, 18 mm in height and 17 mm in width.

The caudal right mandible in the region of the premolar and molar teeth the cortex was within normal limits as was the external shape of the bone itself, however the medullary region of the bone was filled with the same increased opacity material. The distribution was more severe associated with the region of the tooth roots and became less marked towards the ventral mandibular cortex, occupying approximately 2/3rds of the height of the mandible. The lesion was present for approximately 41 mm in length. The vertical ramus of the mandible and the area of the mandible between the two lesions was within normal limits.

Left mandible: A solitary lesion was seen within the rostral left mandible, of similar appearance to that in the right. The lesion was smaller in size and less expansile than the right, however sooth periosteal new bone was a feature. The lesion was again surrounding the incisor tooth root and mental foramen and the medullary region filled with increased opacity material, although some small regions of

APPENDIX F: Computed tomography (CT) report describing lumpy jaw lesions in a red kangaroo (continued)

radiolucency were present within the material on the left (not seen on the right). Similar to the right the cortex within the lesion was reduced in thickness in places, however difficult to define in all areas.

Conclusion:
1. In summary the intraosseous opacity, proliferative appearance with periosteal new bone formation creating expansile or mass type lesions is a feature of these lesions. All 3 lesions on this skull are associated with the bone surrounding the tooth roots. This is similar to that reported.

Dr Jennifer Richardson, BSc, BVMS, MVS, MACVSc, FACVSc(Radiology)